REARING STUDIES OF QUEEN HONEY BEES

A Thesis

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by

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ABSTRACT

Rearing studies of queen honey bees were done using a basic queen rearing system. Measurements were taken of certain external characteristics of both pupae and adult bees.

Three genetic lines were compared and there was a significant difference between the lines for most of the external characteristics measured.

The external morphological characteristics were measured for pupae and adult bees produced from young larvae starved six hours, prior to being placed in queen cells; these were compared with control treatments (young larvae placed directly on a 1:1 royal jelly and distilled water mixture). One of the two experiments showed a significant difference between the control and the starvation treatments for most of the characteristics measured.

A series of experiments was done to study the effect of placing young larvae in one, two, and three day old queen cells. A significant increase in the weight of royal jelly in one, two, and three day old queen cells was found. There was no significant difference for most of the external pupal and adult characteristics measured in the control treatments and those produced from placing young larvae in various locations on the royal jelly in queen cells. A comparison of the pupal and adult external characteristics of bees produced from control treatments, to those of bees produced from placing young larvae in one and two day old queen cells, gave no significant difference between treatments for

most of the characteristics measured. The number of adult bees produced per number of young larvae placed in three day old queen cells was much smaller than the number of adults produced from control treatments.

There was no significant difference in most pupal and adult characteristics measured for bees produced from control treatments and bees produced from placing young larvae in one day old queen cells, ("double grafting"). It was therefore suggested that under normal rearing conditions "double grafting", which requires extra work for the queen producer, does not produce significantly higher quality queens than queens reared from control treatments.

The value of these findings to the beekeeping industry and their relationship to queen - worker differentiation is discussed.

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CHAPTER I

INTRODUCTION

The queen honey bee is an integral part of a normal honey bee colony; the singularity of her presence makes her a subject of special scientific interest. Of equal or greater importance is that the queen performs the major female reproductive role in the colony, and therefore she is essential for the welfare and development of a normal colony. The relationship between the queen's reproductive capacity and honey production in the colony is extremely important to the beekeeping industry itself. The queen also controls the production of queen cells and laying workers by means of her scent and the secretion of "queen substance" from her mandibular glands. Thus a fuller knowledge of queen rearing is desirable in order to better understand her physiology, functions, and performance.

The problem and its importance

The purpose of this study was to show by measurements of pupal and adult external morphological characteristics:

1) The effect on the queens of rearing them from larvae of different genetic stocks,

2) The effect on the queens of rearing them from young larvae starved prior to being placed in queen cells, and

3) The effect on the queens, of rearing them from young larvae in one, two, and three day old queen cells.

The queen rearing industry is an essential part of commercial beekeeping in North America. Every year, thousands of queens are reared and used to found new colonies for honey and wax production. Therefore it has become increasingly necessary to better understand the factors which influence the production of high quality queens, so that recommendations can be made to queen breeders concerning the best queen rearing procedures to follow.

To date little or no data has been published pertaining to whether genetic stock affects the quality of the queens produced, as to whether the possible starvation of larvae prior to placement in queen cells affects the quality of queens produced, or as to whether the placement of a young larva in a one day old queen cell ("double grafting") is necessary to produce high quality queens.

CHAPTER II

QUEEN REARING METHODS

A. Review of literature*

The first scientific account of the natural history of the honey bee was written by Aristotle, (384 - 322 B.C.) who thought that a hive was ruled by a "King" bee. It was not until 1609 that Butler found that the colony was perpetuated by a "Queene".

Swammerdam, in the seventeenth century established conclusively the sex of the queen. In 1771 Schirack showed that the queen could be reared from larvae in worker cells and about the same time Huber became the first man to transfer larvae from worker cells to queen cells and produce queens.

Quinby (1853) advocated the use of swarm cells to produce queens and also described how queen cells could be built by queenless colonies that were given small pieces of comb containing eggs or young larvae from queenright colonies.

Larch (1876) first used the term "grafting" to refer to the substitution of worker larvae for those found in naturally built queen cells.

Some of the more important methods of queen rearing included the Alley, the Miller, the Smith and the Doolittle systems.

The "Alley system", suggested by Henry Alley (1833), involved the rearing of larvae directly from the cells in which the eggs had been

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*Partly from Laidlaw & Eckert (1950).

laid; strong queenless colonies were used for cell builders.

The "Miller system", (1912) was similar to the "Alley system" in that the larvae were allowed to remain in the cells of the worker comb in which they hatched, but instead of cutting up and attaching strips of comb containing eggs and larvae to frames (as in the "Alley system",) he used pieces of wax foundation on which the queen laid. After several days the outer margins of the comb, containing eggs, were cut away and the remaining young larvae and comb placed in a cell building colony.

Smith, (1949), adopted the "Alley System" for large scale queen production. He confined the queen to a small compartment of a larger hive, and forced her to lay on new comb. He then cut the comb into strips which were attached to bars. He used a swarmbox or queenless hive for his starter colony.

Doolittle, about 1870, developed an improved system. He made artificial queen cells of wax, attached twelve queen cell cups to a bar, and placed the bars into a frame the upper portion of which contained comb. He then placed a small amount of royal jelly into each cell cup and transferred larvae under 36 hours of age into each of them. He found that cells built in the second storey of a strong colony (in which the queen was confined to the lower chamber by a queen excluder) gave the best results.

Although Laidlaw and Eckert (1950), described many fundamental queen rearing methods, the opinions, practical experience and research of many people not mentioned have also made valuable contributions to

the queen rearing industry.

Volosevich (1954), published "An Evaluation of Queen Rearing Methods," and classified the methods in the following order according to quality of queens produced; induced swarming, double grafting, queens raised without transfer, and queens raised from grafting once (on honey).

B. Classification of queen rearing methods

Today many of the queen rearing systems make use of the good points of several systems making a definite classification of different queen rearing methods difficult. I have devised the following system for classifying the various methods of queen rearing.

a) The Production of Queens by Natural Methods.

Queen rearing under "natural" conditions involves the rearing of queens without the transferring of individual eggs or larvae to other cells or by cutting up and transferring the comb containing them. Rearing queens under "natural" conditions makes use of the swarming, supersedure or queenless behaviour of honey bees.

b) The Production of Queens by Artificial Methods.

The production of queens by "artificial" methods involves the rearing of queens from the manipulation of individual eggs or larvae. The swarming, supersedure, or queenless behaviour of honey bees may be utilized, but grafting and/or cutting up of the comb by the beekeeper is always involved.

At present there is a good deal of controversy as to whether queens reared under natural conditions or queens reared under artificial

conditions are best for building up colonies for honey production. See Ambruster (1953), Sauvin (1956), Maly (1958), Levicheva (1961) and Bilash (1963).

Sauvin (1956), stated that virgin queens showed no consistent difference in weight according to their rearing conditions during swarming, during supersedure, during an emergency (e.g., when the queen dies), or in commercial methods.

Maly (1958) and Ambruster (1953), both recommended the use of the "swarming impulse" for producing excellent queens.

Levicheva (1961), compared the weights of queens reared under natural and queens reared under artificial conditions:

Method	Number reared	Average weight
Swarm	27	172 ± 2 gms.
Emergency	22	163 ± 2 gms.
Artificial	70	181 ± 1.8 gms.

Altogether 137 queens were reared under artificial conditions and their average weight was 191±0.9 gms. From the data obtained he concluded that queens reared under artificial conditions were superior to queens reared under natural conditions.

Bilash (1963), found experimentally that swarm queens were heavier and had more ovarioles than those reared under artificial conditions.

i) The Two Colony System for Rearing Queens under Artificial Conditions.

The two colony system of queen rearing involves the use of a cell starter and a cell finisher colony. The cell starter is a queenless colony into which the grafted larvae are placed first and accepted. It is here that the building of the queen cell is begun. The cell finisher colonies are strong queenright colonies with the queen confined to the lower box. In these colonies feeding of the larvae is concluded and the cells are capped.

Phillips (1948) used a two colony system and transferred accepted larvae from the cell starter colonies (in which the queen had been temporarily caged) to the cell finisher colonies, 24 hours after grafting.

Holzberlein (1958), worked with units of five colonies consisting of two cell starters, two cell finishers and one nurse colony to supply brood to the cell finishers. He recommended the use of double grafting for producing good queens.

Bilash (1963), also advocated double grafting as being a good method of producing high quality queens.

ii) The One Colony System of Rearing Queens under Artificial Conditions.

Newell (1947), described the "Induced-Supersedure Method" of queen rearing in which the queen is removed from a colony after which the bees rear queens on from 3 to 9 combs.

Gontarski (1952), suggested making a colony queenless, putting in a frame of selected eggs, and letting the bees incubate and raise them. Later, the queen cells were punched out and the queens allowed to emerge.

Shcherbina (1950), dequeened nurse colonies 24 hours before the grafted larvae were placed in them. He found, however, that it was better to dequeen 2 to 4 hours before grafting and thus prevent too many bees from deserting the hive.

C. Miscellaneous queen rearing methods

In the "Stanley system" of queen rearing, (once the initial grafting of the larvae is done), the hive is not affected by adverse weather conditions, because frequent opening of the hive is not necessary to replace or remove cells. This, of course, reduces the amount of labour required as well.

In this system a special "Stanley Patent Cell," is used, consisting of a thin metal tube with one end sharpened; on the other end is fixed a Perspex tip. Through the tube passes a combined cap and plug. Any worker cell cut out by the tube is retained inside it and is pushed through to the tip by the insertion of the cap.

A swarm box with a special swarm box cover made of Perspex is used. The Perspex is perforated so that the "Stanley Patent Cells" can be pushed through the holes.

Brownridge (1953), described what he called the "Morris Board" method of rearing queens. The apparatus enabled flying bees to be directed to the upper box, instead of to the lower brood chamber from which they had come. They could then be isolated in the top box by a sliding board. Thus the queenless top part of the hive had many bees and queen cells

could be started in it on a frame of eggs from the breeder queen. After the cells were started, the board was removed and the bees were allowed access to the remainder of the hive through a queen excluder.

D. Types of queen cell cups

The types of queen cell cups used in artificial queen rearing methods have been studied by Taranov (1949), Vuillaume (1956), Örösi-Pál (1958), Burmistrova (1960), and Inoue and Inoue (1963).

Taranov (1949), found that long cells were detrimental to the larvae, because they could not feed during spinning. He listed cell volumes as follows:

Swarm queen cell volume	824	cu.mm.
Queen cells when a shortage of nectar	728	cu.mm.
Worker cells or larvae when queenless	295-810	cu.mm.
Artificially reared queens	861	cu.mm.

Vuillaume (1956), found that worker bees preferred cells with rounded rather than flat bases, and cylindrical rather than hexagonal shaped cells. The optimum space between cells was 2 cm. and the material used to make the cell cups was of little importance. He found that acceptance was not inhibited by the presence of the queen, and was better if the cells were placed in the hive overnight before grafting; presumably to acquire the hive odor. He found (1957) that if he used a short cup with too wide an opening the bees partly closed it and if a long cup was used the bees built wax on the inside. He also stated that the percentage acceptance was affected by the shape of the queen cell cup.

Örösi-Pál (1958), stated that more queens are obtained from grafted cups than from hung worker cells.

Burmistrova (1960), compared normal queen cell cups with reduced or shortened worker cells, by grafting 1 to 1½ day old larvae into both. He removed the royal jelly and found 74.4 mgs. in the normal queen cell cups and 31.6 mgs. in worker cells, the sides of which had been cut down prior to grafting. Queens from normal cells were heavier (145 mgs.) than queens from "cut down" worker cells (137 mgs.) and they also possessed a greater number of ovarioles (241 to 197).

Inoue and Inoue (1963), studied the utilization of plastic queen cell cups for royal jelly production and noted acceptance and the mean weight of royal jelly per cell as follows:

	Plastic queen cell cups	Wax queen cell cups
Acceptance	90.3%	75.8%
Average weight/cell	335.5 mgs.	364.6 mgs.

E. Queen rearing methods used in this study

A single box breeder colony, (Figure 1,1), in which the queen was confined to a one frame excluder (Figure 1,3), was used. The queen cells were started and finished in the same cell builder colonies, (Figure 1,2). These were queenright colonies with the queen confined to the bottom box by an excluder. The colonies were very populous and up to five boxes above the excluder were present at the end of the season. Frames, containing larvae for grafting, were removed from the breeder colonies and placed in grafting stands (Figure 2,1). Larvae, under 6 hours old

(Chapter V page 32), were lifted with a grafting hook from a comb and transferred into plastic queen cell cups which contained a small drop of 1:1 mixture of royal jelly and distilled water (Figure 2,2). The frames containing the queen cell cups were placed in the top boxes of the cell builder colonies, with capped brood on both sides of the frame. The frames were removed from the hives when the cells were capped (Figure 2,3), and the cells placed in an incubator kept at 32°C and approximately 90% relative humidity. Each cell was removed from the frame and placed hanging downwards in a glass vial large enough in diameter to allow the wax tip of the cell but not the plastic base to enter the vial. The adult bees were removed from the vials and preserved in F.A.A. solution in smaller vials, soon after emergence.

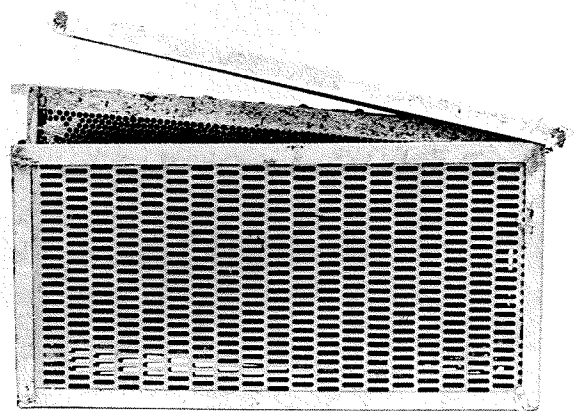

FIGURE 1

QUEEN REARING EQUIPMENT

1. Breeder colony

2. Cell builder colony

3. One frame queen excluder

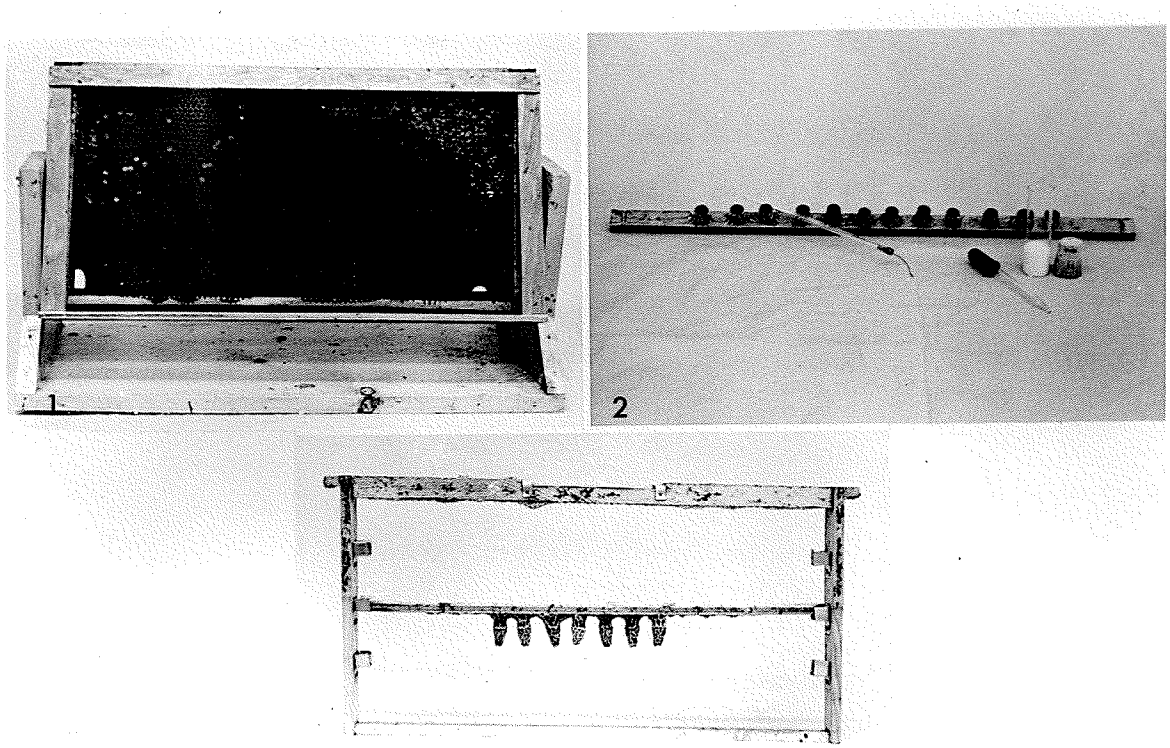

FIGURE 2

QUEEN REARING EQUIPMENT

1. Grafting stand containing brood frame.

2. Queen cell cups, royal jelly and grafting hook.

3. Queen rearing frame containing capped queen cells.

CHAPTER III

MEASUREMENTS

The honey bee has two female castes, the worker and the queen. Sometimes, bees intermediate in form between worker and queen are found. (Rhein 1933, 1951; Komarov 1935; Weaver 1957; Smith 1959). A review of characteristics used in differentiating between these forms follows.

A. WEIGHTS

Weights obtained with larvae that had just completed their cocoons or with mature pupae were found more satisfactory criteria than adult weights for differentiating between workers, intercastes, and queens (Jay 1964).

B. THE HEAD

Vagt (1955), Lukoschus (1956), and Naulleau (1960), found that the head of the queen was somewhat round as compared to the more triangular head of the worker.

Vagt (1955), found that if the width and length of the head were taken as a ratio, queen and worker head measurements differed. He was able to classify adults as perfect or partial queens on this basis.

Lukoschus (1956), and Naulleau (1960), observed that the ocelli of the worker were on the vertex of the head, but on the frons for the queen.

C. THE ABDOMEN

Smith (1959), stated that the abdomen of the queen was relatively long and slender compared with that of the worker.

Weaver (1955, 1957), used abdominal breadth and length as an aid in classifying workers, intercastes, and queens.

Lukoschus (1956), measured length, height and width of the abdomen and obtained the following results:

Queen:	13-18;	5;	5.5 mm.
Worker:	8-10;	3-4;	4 mm.

D. THE APPENDAGES

1. Appendages of the head

a) The Proboscis (tongue length)

Rhein (1933), stated that a short tongue was an important queen characteristic. He measured it from the end point of the mandible to its distal end.

Smith (1954), and Weaver (1955, 1956, 1957), also used proboscis length as a criteria for classifying workers, intercastes and queens. Smith (1959), found, however, that the length of the tongue was the most variable characteristic measured and that it was not always a reliable indicator of the degree of worker-like or queen-like differentiation. He found that measurements of the tongue were most easily and accurately measured in the pupal stage and he measured them from the tips of the mandibles to the distal ends of the tongues. Jay (1964), has also used

this measurement for classifying worker, intercaste, and queen pupae.

b) The Mandibles

Rhein (1933), Komarov (1935), Lukoschus (1956), Snodgrass (1956), Smith (1959), and Naulleau (1960), described the mandibles of the queen as having a large notch or toothed projection.

Komarov (1935), Lukoschus (1956), and Smith (1959), described the mandibles of the worker as being rounded and lacking a notch or toothed projection. Naulleau (1960), and Snodgrass (1956), described the worker mandible as being large at the base, narrowing, and then becoming wider again at the extremity, which was rounded. Smith (1959), and Naulleau (1960), described intermediate forms as having slightly toothed mandibles.

2. Appendages of the thorax

a) The Metathoracic legs

Rhein (1933), Komarov (1935), Vagt (1955), Snodgrass (1956), and Smith (1959), stated that the hind leg of the worker differed from that of the queen by the presence of a pollen basket and pollen brush. Rhein (1933) and Lukoschus (1956), described the pollen brush of the worker as being made up of ten rows of long bristles, but Snodgrass (1956), and Smith (1959), found that the first segment of the tarsus had nine rows of bristles which formed the pollen brush.

Vagt (1955), and Weaver (1957), measured the ratio of the length to width of the tarsus. Vagt (1955), found that the mean value of this

ratio for swarm queens was 2.30268 and for workers 1.77639. He found that the index from larva $\frac{1}{2}$ to 1 day old, which produced queens, was 2.29865 and 2.28578, respectively.

Naulleau (1960), has shown the difference between the hind legs of workers and queens in diagrams.

E. OTHER CHARACTERISTICS

A number of other characteristics were reviewed but were not chosen as criteria for use in this thesis.

Weaver (1955, 1957), used abdominal breadth as a criteria for classifying workers, intercastes and queens, and Lukoschus (1956), measured the height and width of the abdomens of workers and queens.

Kresok (1952), defined body size by using the length and width of the third abdominal tergite. Bornus (1960), took the sum of the width of the third and fourth abdominal tergites as a measure of body size, and gave correlation coefficients between this and other characteristics (i.e., the width of the fourth sternite, the width of the forewing, the length of the forewing, the cubital index, the length of glossa, and the area of wax plates on the fourth sternite).

Koshevnikoff (1905), cited by Komarov (1935), and Naulleau (1960), compared the sixth abdominal sternite of the queen with that of the worker. Komarov (1935), examined the third and fourth abdominal sternites of the queen and studied the wax glands of workers and intermediates. Lukoschus (1956), measured the width and length of the third abdominal sternites of queens and workers.

Snodgrass (1956), Weaver (1955, 1957), Smith (1959), and Naulleau (1960), described the differences in the number of barbs and the shape of the stings, between the worker and the queen.

F. MEASUREMENT USED IN THIS THESIS

The external morphological characteristics of the honey bees obtained in this study were measured with a binocular microscope having an eye piece fitted with a linear scale. The characteristics measured were chosen on the basis of simplicity of measurement, degree of accuracy obtained, and their value as indicators of dimorphic differentiation. The measurements used are listed below.

Pupal measurements

1. Length of tongue:

This was measured from the base of the mandibles to the tip of the tongue. Figure 3,1.

2. Length of abdomen -- side view:

This was measured from the small anterior indentation of the second true abdominal tergite:

a) along a line parallel with the axis of the body, to a point where a perpendicular from the tip of the abdomen (exclusive of the sting shaft) intersects. Figure 3,2.

b) along a straight line to the tip of the abdomen (exclusive of the sting shaft). Figure 3,2.

3. Length of pupa:

The sum of the length, from the small anterior indentation of the second true abdominal segment to the frons of the head, and the abdominal measurement (a). Figure 3,2.

4. Weight of pupa:

This was measured in milligrams about 3 days after pupation. The number of days after pupation was estimated by the appearance and color of the pupae (Jay 1962).

Adult measurements

All adult bees were placed in small vials and preserved in F.A.A. solution soon after emergence.

5. The head -- anterior view:

a) Width: this was measured across the widest part of the head from one lateral edge (parietal area) of the head capsule to the other. Figure 4,1.

b) Length: this was measured from the vertex of the head to the distal edge of the labrum. Figure 4,1.

6. The basitarsus:

The large first segment of the tarsus of the hind leg, of which the inner surface of the right hind basitarsus was measured. Figure 4,2.

a) Length: this was measured from the outer tip of the auricle, along the outer portion of the basitarsus, to its most distal point. Figure 4,2.

b) Width: this was a perpendicular measurement from one margin across to the other, at the widest part of the basitarsus which was approximately one half to three fifths of the total length from the proximal end. Figure 4,2.

Other data recorded for specific tests included larval mortality, the time of cell capping, the length of the cell, and the total developmental time.

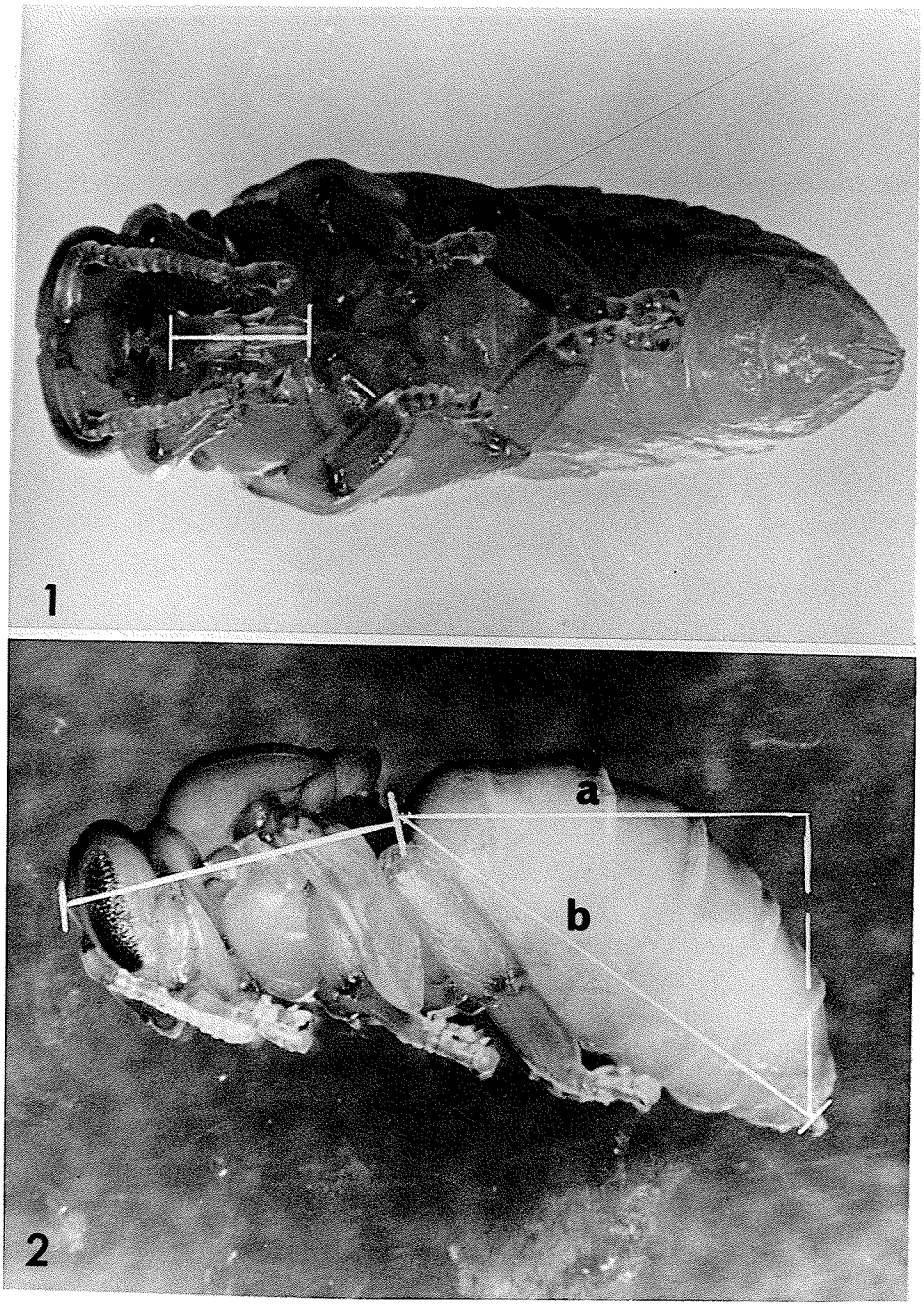

FIGURE 3

PUPAL MEASUREMENTS

1. Length of tongue
2. a. length of abdomen (a^1)
   b. length of abdomen (b^2)
   and total length of pupa

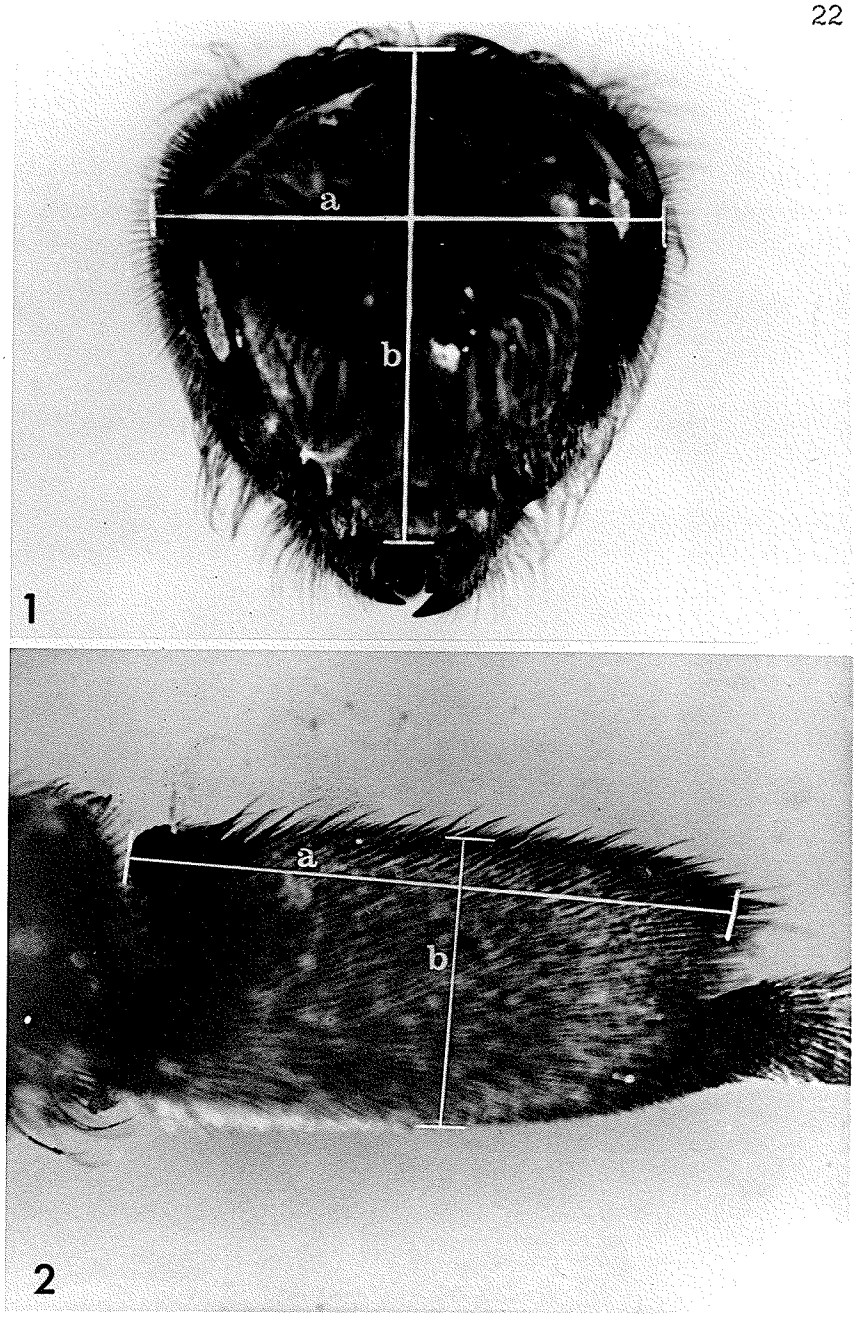

FIGURE 4

ADULT MEASUREMENTS

1. Head
   a. width
   b. length

2. Basitarsus
   a. length
   b. width

CHAPTER IV

THE IMPORTANCE OF GENETIC LINES IN THE PRODUCTION OF QUEENS

INTRODUCTION

Biometrical studies of queen honey bees were conducted to compare the external morphological characteristics of queens produced from larvae of different genetic lines.

Methods and results

Queens produced from larvae of A, B and C genetic lines were compared. A and B were separate genetic lines produced from crossing inbred Italian with inbred Carniolan stocks, and line C was an Italian hybrid queen, selected arbitrarily from one of the packages of bees received in the spring.

The basic queen rearing method described in Chapter II, page 10 was used. Four experiments were conducted comparing lines A to B (two replicates), A to C, and B to C; the larvae for all experiments were placed in one hive, where they remained until the cells were capped. Each experiment consisted of two treatments, six larvae per treatment, with extra larvæ grafted to serve as replacements in the event of early mortality.

The results are shown on Tables I-VI, inclusive. The means, standard deviations, and T test probability values were calculated.

Experiments 1 and 4 were combined; there was a significant difference between lines A and B for the length of tongue, weight of pupa, width of head, and length of head. In experiment 2 there was a significant difference between the length of body, length of tongue, length of abdomen (b^2), weight of pupa and length of head of lines B and C. A significant difference was found between lines A and C for the weight of pupa, width of head, and length of head in experiment 3, (See Table VI.).

Discussions and conclusions

There was no significant difference between lines in any single experiment, e.g., A to B (two replicates combined), A to C, or B to C, for length of abdomen (a^1), length of basitarsus, width of basitarsus, and length of cell, suggesting that these measurements might be fairly constant for most genetic strains. In every case, where a significant difference was found between genetic lines, the average pupal and adult measurements of C were greater than B, and those of B and C were greater than A genetic lines, (i.e., C > B > A). See Table VI. (EXCEPTION i)

The results appear to indicate that size differences do exist between some genetic lines and that pupal weight is one of the best criteria for separating genetic lines (Table VI.). The results show highly significant T test probability values for pupal weight between the three strains tested; of importance too is that the measurements can be made early in the development of the queen.

(i) B>C FOR HEAD LENGTH.

TABLE I

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM LARVAE OF A AND B GENETIC LINES

Experiment	Bee no.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal Index (L/W)	Length of capped cell (mm)	Length of development (hrs.)
	1	15.60	2.44	9.26	8.78	256	3.67	3.61	1.02	2.52	1.20	2.10	13	303.0±4.5
	2	15.60	2.60	9.43	8.61	268	3.80	3.67	1.03	2.52	1.20	2.10	16	285.5±2.0
Line A	3	15.93	2.76	9.10	8.61	254	3.86	3.67	1.05	2.56	1.24	2.06	14	279.0±4.5
	4	15.60	2.68	9.43	8.61	269	3.80	3.67	1.03	2.52	1.24	2.03	14	279.0±4.5
	5	15.76	2.44	8.61	8.45	267	3.86	3.67	1.05	2.52	1.24	2.03	14	303.0±4.5
	6	15.93	2.60	9.26	8.94	245	3.80	3.61	1.05	2.56	1.24	2.06	13	285.0±1.5
1.	Mean	15.74	2.59	9.18	8.67	259.8	3.80	3.65		2.53	1.23		14.0	
	Standard Deviation	.15	.12	.28	.15	8.86	.07	.03		.02	.02		1.00	
	1	16.09	2.68	9.10	8.78	281	3.92	3.80	1.03	2.64	1.24	2.13	14	303.0±4.5
	2	16.41	2.60	9.44	8.94	281	3.99	3.80	1.05	2.68	1.28	2.09	15	303.0±4.5
Line B	3	16.09	2.60	9.10	8.78	275	3.86	3.80	1.02	2.64	1.24	2.13	14	303.0±4.5
	4	16.41	2.68	9.44	9.10	284	3.99	3.80	1.05	2.64	1.24	2.13	14	303.0±4.5
	5	16.09	2.76	8.94	8.78	275	3.92	3.80	1.03	2.72	1.28	2.13	15	303.0±4.5
	6	16.25	2.68	9.10	8.94	280	3.92	3.80	1.03	2.60	1.24	2.10	14	303.0±4.5
	Mean	16.22	2.67	9.19	8.89	279.3	3.93	3.80		2.65	1.25		14.3	
	Standard Deviation	.15	.06	.19	.12	3.30	.05	0.00		.04	.02		.47	

TABLE II

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM LARVAE OF B AND C GENETIC LINES

Experiment	Bee no.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
	1	15.76	2.76	8.78	8.61	277	3.92	3.80	1.03	2.56	1.20	2.13	14	286.25±1.25
	2	15.93	2.60	9.10	8.78	273	4.05	3.80	1.07	2.64	1.20	2.20	14	278.5±4.5
Line B	3	15.93	2.68	9.26	8.61	274	3.92	3.80	1.03	2.68	1.28	2.09	15	289.75±1.25
	4	16.09	2.76	9.10	8.94	266	3.99	3.73	1.07	2.52	1.24	2.03	13	278.5±4.5
	5	16.09	2.60	9.26	8.61	275	3.99	3.73	1.07	2.64	1.24	2.13	14	284.0±1.0
	Mean	15.85	2.61	9.10	8.64	267.8	3.97	3.77		2.61	1.23		14.0	
	Standard Deviation	.12	.02	.18	.13	3.74	.05	.04		.06	.03		.63	
2.														
	1	15.76	2.68	9.10	8.60	276	3.86	3.61	1.07	2.64	1.20	2.20	14	289.75±1.25
	2	16.41	2.76	9.43	9.26	290	3.92	3.73	1.05	2.72	1.20	2.27	14	284.0±1.0
Line C	3	16.25	2.76	9.10	8.94	278	3.86	3.73	1.03	2.60	1.20	2.17	14	278.5±4.5
	4	16.25	2.68	9.75	8.94	297	3.99	3.73	1.07	2.64	1.20	2.20	15	302.0±4.5
	5	16.41	2.84	9.26	9.10	294	3.92	3.67	1.07	2.64	1.24	2.13	13	302.0±4.5
	6	16.41	2.68	9.26	9.10	308	3.99	3.73	1.07	2.56	1.20	2.13	14	289.75±1.25
	Mean	16.25	2.73	9.32	8.99	290.5	3.92	3.70		2.63	1.21		14.0	
	Standard Deviation	.23	.06	.22	.21	11.01	.06	.05		.05	.01		.55	

TABLE III

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM LARVAE OF A AND C GENETIC LINES

Experiment	Bee no.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/LO	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
LINE A	1	16.41	2.60	9.28	9.10	267	3.86	3.67	1.05	2.56	1.16	2.21	14	304.0±4.5
	2	16.58	2.44	9.75	9.43	278	3.86	3.67	1.05	2.56	1.20	2.13	16	304.0±4.5
	3	16.58	2.60	9.59	9.26	283	3.80	3.67	1.04	2.68	1.16	2.31	14	304.0±4.5
	4*						3.73	3.61	1.03	2.56	1.20	2.13	15	278.5±4.5
	5	16.41	2.76	9.43	9.10	270	3.86	3.67	1.05	2.52	1.24	2.03	15	304.0±4.5
	6	16.25	2.52	9.59	9.10	265	3.73	3.67	1.02	2.56	1.20	2.13	15	304.0±4.5
	Mean	16.45	2.58	9.53	9.20	272.6	3.81	3.66		2.57	1.19		14.8	
	Standard Deviation	.12	.11	.16	.13	6.83	.06	.22		.05	.03		.71	
LINE C	1	16.25	2.44	9.59	9.10	285	3.80	3.67	1.04	2.60	1.16	2.24	15	304.0±4.5
	2	16.90	2.44	10.24	9.59	305	3.92	3.80	1.03	2.76	1.20	2.30	15	328.0±4.5
	3	16.90	2.60	10.08	9.75	292	3.92	3.80	1.03	2.60	1.20	2.17	15	304.0±4.5
	4	16.58	2.44	9.91	9.26	281	3.92	3.73	1.05	2.56	1.20	2.13	15	304.0±4.5
	5	16.90	2.44	10.08	9.59	286	3.86	3.73	1.03	2.64	1.16	2.28	14	304.0±4.5
	6	16.58	2.44	9.59	9.29	290	3.92	3.67	1.07	2.60	1.16	2.24	14	310.0±1.5
	Mean	16.69	2.47	9.92	9.43	289.8	3.89	3.73		2.63	1.18		14.7	
	Standard Deviation	.24	.06	.25	.23	7.66	.46	.07		.04	.02		.47	

* Ready to emerge before pupa could be measured.

TABLE IV

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM LARVAE OF A AND B GENETIC LINES

Experiment	Bee no.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
Line A	1	16.90	2.68	10.08	9.75	272	3.80	3.67	1.04	2.64	1.20	2.20	16	304.0±4.5
	2*						3.92	3.80	1.03	2.60	1.20	2.17	14	284.0±4.5
	3*						3.86	3.67	1.05	2.52	1.28	1.97	15	284.0±4.5
	4	16.25	2.44	9.91	9.26	270	3.73	3.54	1.05	2.60	1.24	2.10	15	284.0±4.5
	5	16.74	2.44	10.40	9.75	251	3.80	3.61	1.05	2.56	1.16	2.21	14	284.0±4.5
	6	16.25	2.44	9.59	8.94	273	3.73	3.67	1.02	2.68	1.24	2.16	15	304.0±4.5
Mean		16.54	2.50	10.00	9.43	266.5	3.81	3.66		2.60	1.22		14.8	
Standard Deviation		.29	.10	.29	.34	9.01	.07	.08		.05	.04		.90	
4.														
Line B	1	16.90	2.44	10.56	10.08	265	3.86	3.73	1.03	2.56	1.28	2.00	16	300.5±6.0
	2	16.58	2.60	9.91	9.43	270	3.92	3.67	1.07	2.56	1.16	2.21	17	291.0±2.0
	3	16.58	2.84	9.75	9.10	282	4.05	3.86	1.05	2.60	1.24	2.10	17	204.0±4.5
	4	16.25	2.68	9.59	8.94	280	3.99	3.80	1.05	2.68	1.24	2.16	15	204.0±4.5
	5	16.41	2.68	10.08	9.26	276	3.86	3.73	1.03	2.52	1.20	2.10	15	204.0±4.5
	6	16.41	2.76	9.75	8.94	300	4.05	3.92	1.03	2.72	1.28	2.13	14	204.0±4.5
Mean		16.52	2.66	9.94	9.29	278.8	3.91	3.79		2.61	1.23		15.7	
Standard Deviation		.20	.13	.32	.39	11.08	.08	.09		.07	.04		.35	

* Ready to emerge before pupa could be measured.

TABLE V

A SUMMARY OF LARVAL MORTALITY AND TIME OF CAPPING OF CELLS OF QUEENS

REARED FROM LARVAE OF A, B, AND C GENETIC LINES

Experiment		No. of larvae per treatment	Larval mortality at 1st.check (hrs)	Total larval mortality between grafting and capping	Total no. of larvae replaced	No. of cells capped at the times indicated (hrs)			Total No. of adults produced
1	Line A	6	0 (21)	4	4	6(103.5)			6
	Line B	6	0 (21)	0	0	6(103.5)			6
2	Line B	6	3 (21.5)	3	3	1(93.5)	6(98)	6(117)	5
	Line C	6	1 (21.5)	1	1	0(93.5)	3(98)	6(117)	6
3	Line A	6	0 (21.5)	1	1	2(93.5)	3(98.5)	6(117.5)	6
	Line C	6	0 (21.5)	0	0	0(93.5)	3(98.5)	6(117.5)	6
4	Line A	6	0 (21.5)	0	0	4(95.5)	4(98.5)	6(119.5)	6
	Line B	6	0 (21.5)	0	0	1(95.5)	5(98.5)	6(119.5)	6

TABLE VI

T TEST PROBABILITY VALUES FROM COMPARING DIFFERENT GENETIC LINES

Experiment	Genetic lines compared	Length of body	Length of tongue	Length of abdomen a[1]	Length of abdomen b[2]	Weight of pupa	Head width	Head length	Basitarsus length	Basitarsus width	Length of cell
1 and 4 (combined)	A-B	N.S.	2.37 P<0.05 B+	N.S.	N.S.	4.09 P<0.01 B+	5.05 P<0.01 B+	5.39 P<0.01 B+	N.S.	N.S.	N.S.
	Df = 20						Df=22				
2	B-C	3.16 P<0.05 C+	2.75 P<0.05 C+	N.S.	2.97 P<0.05 C+	3.98 P<0.01 C+	N.S.	2.55 P<0.05 B+	N.S.	N.S.	N.S.
	Df = 9										
3	A-C	N.S.	N.S.	N.S.	N.S.	3.53 P<0.01 C+	2.42 P<0.05 C+	2.72 P<0.05 C+	N.S.	N.S.	N.S.
	Df = 9						Df=10				

Legend: N.S. = Non Significant
Df = Degrees of freedom
\+ = Genetic line having the largest mean within an experiment.

CHAPTER V

THE EFFECT ON QUEENS WHEN REARED FROM STARVED AND NON-STARVED LARVAE

INTRODUCTION

Stunted or dwarf worker bees are very common in package bees in the spring. They develop this way due to poor food supplies, especially lack of pollen caused by poor weather conditions. Adverse weather conditions also cause a shortage of food for feeding young larvae, used for grafting in commercial queen rearing. Therefore, a study was made to determine the effect of starvation on young larvae prior to grafting as shown by the external morphological characteristics of the pupae and adult bees, produced from them.

Methods and results

Several techniques for starving young larvae were tested. Larvae, less than 6 hours old (see page 32), were placed in an incubator kept at 32°C and about 90% R.H. for the duration of a six hour starvation period.

In preliminary tests the effects of starving larvae on a dry surface was first tested, e.g., wax and blotting paper. Both treatments resulted in extremely high mortalities. The effect of starving larvae on the surface of moist blotting paper was next tested and this treatment gave a somewhat lower mortality rate.

In experiments 1, 2 and 3, (Table VIII), in which larvae were starved on moist blotting paper, such a high mortality level was observed

that it was not considered a desirable method. When a water soluble dye was mixed in the water used to moisten the blotting paper, it was found that the larvae were ingesting the water. It was thought that the ingested water might affect the viability of the larvae and/or possibly the queen - worker differentiation process, through food dilution.

Therefore, tests 1 and 2 were conducted to compare the mortality of larvae starved on moist blotting paper to that of larvae starved on a strip of dry gauze, stretched over a petri dish filled with distilled water 1/3 to 1/4 of an inch from the top. The dry gauze prevented the larvae from ingesting water and the nearness of the water surface probably maintained a high humidity around the larvae. Table VII shows (tests 1 and 2 combined), that significantly fewer larvae died ($P < 0.01$) prior to grafting when larvae were starved on dry gauze in high humidity rather than on the moist blotting paper.

Experiments 5, 6 and 7 were therefore done in which larvae were starved on a strip of dry gauze; the control larvae for these experiments were less than $3\frac{1}{2}$, 4, and $3\frac{1}{2}$ hours old respectively when grafted, and the larvae to be starved were the same respective ages when starvation commenced. The starved larvae were grafted 6 hours after the control larvae, and due to the starvation period, they were $9\frac{1}{2}$, 10, and $9\frac{1}{2}$ hours old respectively when grafted.

The maximum possible age of the larvae was determined by one of two methods. If a large number of eggs and few larvae were present on

a breeder comb, all the larvae were removed, and the maximum age of any new larvae that hatched was therefore known at any given time. If the comb contained too many larvae to discard in a reasonable period of time, a deep indentation was cut on the comb encircling all the larvae. The maximum age of any larvae outside the indentation ring, that later hatched, was then known. Despite the fact that the starvation experiments were performed in late summer when the queen usually lays few eggs, there was little difficulty in obtaining 72 larvae under $3\frac{1}{2}$ hours of age for an experiment. Therefore, there was very little error in the selection of larvae under 6 hours of age for all other experiments presented in this thesis.

The means, standard deviations and T test probability values were calculated (Tables IX and X), for experiments 4 and 6, with experiment 5 being excluded because of the very low number of adults produced from the starvation treatment. In experiment 4 (Table XI), there was a significant difference ($P \leq 0.01$) between the treatments in all measurements except the width and length of head and the width of basitarsus, while in experiment 6 (Table XI), there was no significant difference between any of the measurements of the starved and control larvae.

Discussions and conclusions

Table VII shows that less larval mortality occurs prior to grafting when larvae are starved on dry gauze in close proximity to a water surface than when larvae are starved on moist blotting paper ($P \leq 0.01$).

The ingestion of water by larvae starved on moist blotting paper probably contributes to the high larval mortality.

The difference in T test probability values (p) between experiments 4 and 6, prevent one from drawing definite conclusions about the effect of starvation on young larvae prior to grafting, as shown by the queens produced. Different genetic lines and different hives were used in experiments 4 and 6, and these may have affected the results. The significant differences ($P < 0.01$) obtained between starved and control treatments for six characteristics in experiment 4, suggests, however, that further work is required before the effect of starvation can be ignored by commercial queen producers.

TABLE VII

A COMPARISON OF LARVAL MORTALITY WHEN LARVAE WERE STARVED ON DRY GAUZE ABOVE A WATER SURFACE AND ON MOIST BLOTTING PAPER

Test	Starvation treatment	No. of larvae per treatment	Larval mortality after 6 hrs starvation
1	moist blotting paper	12	9
	gauze	12	1
2	moist blotting paper	12	9
	gauze	12	0

Note: The Chi square probability value for test 1 and 2 combined, comparing moist blotting paper and gauze: ($P < 0.01$).

TABLE VIII

MORTALITY OF LARVAE WHEN STARVED 6 HOURS ON VARIOUS SUBSTRATA

Experiment	Treatment	No. larvae per treatment	Genetic line	Maximum larval age (hrs.) of controls grafted and larvae prior to starvation	Larval mortality at 1st. check	(hrs.after grafting)	Total larval mortality between grafting and capping	Total no. of adults produced
1. moist blotting paper	starve	36	A	6	13	(19)	23	12
	control	36	A	6	4	(19)	4	23
2. moist blotting paper	starve	36	A	6	30	(18.5)	33	2
	control	36	A	6	7	(18.5)	8	26
3. moist blotting paper	starve	36	B	3½	27	(45.5)	27	9
	control	36	B	3½	8	(39.5)	8	21
4. gauze	starve	36	B	3½	17	(22)	21	14
	control	36	B	3½	5	(16)	9	27
5. gauze	starve	24	A	4	18	(23.5)	20	2
	control	24	A	4	6	(17.5)	10	12
6. gauze	starve	36	A	3½	16	(21)	29	6
	control	36	A	3½	6	(15)	10	26

TABLE IX

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM STARVED AND NON-STARVED LARVAE

Experiment	Treatment	Bee no.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal Index (L/W)	Length of capped cell (mm)	Length of development (hrs)
4*	Starvation	1⊖	14.95		8.45	8.13	223	3.80	3.61	1.05	2.32	1.20	1.93	15	361.5±4.5
		2	16.90	2.68	9.75	9.43	301	3.92	3.73	1.05	2.36	1.12	2.11	17	362.0±4.5
		3	16.09	2.52	9.26	8.78	281	3.73	3.54	1.05	2.48	1.16	2.14	13	362.0±4.5
		4	16.09	2.60	9.10	8.94	274	3.80	3.67	1.04	2.40	1.16	2.07	16	362.0±4.5
		5	16.58	2.60	9.43	9.10	277	3.92	3.61	1.09	2.52	1.16	2.17	14	348.±6.0
		6	16.41	2.44	9.59	9.10	282	3.80	3.54	1.07	2.48	1.16	2.14	17	351.0±6.0
		7	16.25	2.44	9.26	9.10	264	3.86	3.67	1.05	2.48	1.16	2.14	13	362.0±4.5
		8	15.60	2.44	8.78	8.61	246	3.80	3.54	1.07	2.32	1.16	2.00	16	362.0±4.5
		9⊖	16.25		9.43	8.94	272	3.86	3.61	1.07	2.36	1.16	2.03	16	366.75±0.25
		10	15.11	2.60	8.45	8.13	223	3.86	3.54	1.09	2.16	1.12	1.93	14	
		11	15.60	2.20	8.61	8.29	227	3.80	3.48	1.09	2.28	1.20	1.90	16	
		12⊖	15.60		8.94	8.29	227	3.80	3.54	1.07	2.36	1.08	2.19	15	362.0±4.5
		13	15.93	2.44	9.43	8.78	275	3.92	3.67	1.07	2.48	1.16	2.14	15	
		14	16.41	2.52	9.43	9.10	277	3.86	3.73	1.03	2.52	1.20	2.10	16	332.5±2.5
	Mean		15.98	2.50	9.14	8.77	260.6	3.84	3.61		2.39	1.16		15.2	
	Standard Deviation		.65	.12	.40	.40	25.17	.05	.08		.10	.03		1.26	

* Genetic line = B.
⊖ Tongue bent.

continued...

TABLE IX (continued)

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
4*	Control	1	16.58	2.68	9.75	9.10	301	4.05	3.80	1.07	2.56	1.20	2.13	18	325.5±4.5
		2	16.41	2.52	9.43	9.10	283	3.80	3.61	1.05	2.52	1.12	2.25	17	325.5±4.5
		3	16.74	2.52	9.43	9.26	293	3.86	3.61	1.07	2.52	1.20	2.10	16	325.5±4.5
		4	16.25	2.60	9.26	8.94	280	3.92	3.73	1.05	2.52	1.20	2.10	15	316.75±2.75
		5	16.25	2.68	9.26	8.94	287	3.86	3.67	1.05	2.56	1.20	2.13	15	325.5±4.5
		6	16.25	2.76	9.43	8.94	295	3.99	3.73	1.07	2.64	1.16	2.28	17	325.5±4.5
		7	16.58	2.60	9.75	9.43	263	3.86	3.61	1.07	2.60	1.16	2.24	17	325.5±4.5
		8	16.58	2.60	9.59	9.26	276	3.80	3.54	1.07	2.52	1.20	2.10	17	325.5±4.5
		9	16.90	2.52	10.40	9.91	271	3.86	3.61	1.07	2.48	1.20	2.07	17	309.0±2.0
		10	16.74	2.60	9.75	9.43	277	3.80	3.61	1.05	2.48	1.16	2.14	17	325.5±4.5
		11	16.41	2.60	9.43	9.10	272	3.92	3.67	1.07	2.60	1.20	2.17	15	325.5±4.5
		12	15.93	2.44	9.10	8.61	269	3.80	3.61	1.05	2.48	1.16	2.14	17	337.75±0.25
		13	16.58	2.60	9.75	9.26	264	3.86	3.54	1.09	2.48	1.12	2.21	16	325.5±4.5
		14	16.90	2.52	10.08	9.59	266	3.80	3.54	1.07	2.56	1.16	2.21	16	325.5±4.5
		15	16.41	2.52	9.59	9.26	290	3.86	3.67	1.05	2.56	1.20	2.13	17	325.5±4.5
		16	16.74	2.60	10.08	9.75	264	3.80	3.54	1.07	2.44	1.16	2.10	16	316.25±3.25
		17	16.90	2.60	10.24	9.75	286	3.92	3.73	1.05	2.52	1.20	2.10	16	316.25±3.25
		18	16.74	2.52	10.08	9.59	289	3.80	3.61	1.05	2.52	1.16	2.17	17	332.5±2.5
		19	16.58	2.68	10.24	9.43	290	3.92	3.67	1.07	2.52	1.20	2.10	17	316.25±3.25
		20	16.09	2.60	9.26	9.10	256	3.86	3.61	1.07	2.44	1.16	2.10	15	316.25±3.25
		21	16.09	2.60	9.43	8.78	272	3.86	3.54	1.09	2.56	1.16	2.21	18	335.75±1.75
		22	17.06	2.68	9.91	9.43	319	3.99	3.80	1.05	2.56	1.12	2.29	16	337.75±0.25
		23[e]						3.86	3.80	1.02	2.48	1.16	2.14	18	302.5±4.5
		24	16.41	2.60	9.59	9.10	272	3.86	3.61	1.07	2.56	1.20	2.13	17	325.5±4.5
		25	16.58	2.60	9.91	9.43	273	3.86	3.61	1.07	2.48	1.20	2.07	16	316.25±3.25
		26	17.06	2.60	10.89	10.08	289	3.92	3.73	1.05	2.56	1.20	2.13	17	312.0±1
		27	16.58	2.60	9.59	9.43	261	3.80	3.61	1.05	2.48	1.12	2.21	17	325.5±4.5
		Mean	16.55	2.59	9.74	9.31	279.2	3.87	3.64		2.53	1.17		16.6	
		Standard Deviation	.63	.05	.41	.34	14.14	.07	.08		.05	.03		.87	

*Genetic line = B.

[e]Wings blown before pupal measurements could be taken.

TABLE X

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM STARVED AND NON-STARVED LARVAE

Experiment	Treatment	Bee no.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal Index (L/W)	Length of capped cell (mm)	Length of development (hrs)
6*	starvation	1	16.09	2.60	9.26	8.94	273	3.73	3.61	1.03	2.56	1.20	2.13	20	332.5±1.0
		2	16.25	2.60	9.43	9.10	281	3.73	3.61	1.03	2.36	1.16	2.03	19	317.25±2.25
		3	16.09		8.94	8.61	226	3.61	3.35	1.08	2.32	1.32	1.76	20	
		4	16.74	2.60	10.08	9.43	298	3.73	3.48	1.07	2.36	1.16	2.03	19	351.0±4.5
		5	15.93	2.60	9.59	8.61	268	3.67	3.54	1.04	2.48	1.12	2.21	16	337.25±1.75
		6	16.41	2.60	9.43	9.10	293	3.73	3.54	1.05	2.68	1.20	2.23	19	333.5±1.0
		Mean	16.25	2.60	9.46	8.97	273.2	3.70	3.52		2.46	1.19		18.8	
		Standard Deviation	.26	.00	.35	.29	23.53	.05	.09		.13	.06		1.34	
	control	1	16.25	2.84	9.57	8.94	275	3.73	3.61	1.03	2.44	1.20	2.03	19	314.0±1.0
		2	16.41	2.60	9.26	9.10	272	3.67	3.48	1.05	2.48	1.16	2.14	16	308.5±4.5
		3	15.60	2.60	9.43	8.78	264	3.73	3.48	1.07	2.40	1.16	2.07	19	317.25± 2.25
		4	15.93	2.60	9.10	8.61	257	3.73	3.54	1.05	2.40	1.08	2.22	19	308.5±4.5
		5	16.09	2.76	9.26	8.78	261	3.73	3.54	1.05	2.48	1.20	2.07	19	317.25±2.25
		6	16.41	2.68	9.43	9.10	279	3.80	3.61	1.05	2.48	1.12	2.21	18	308.5±4.5
		7	16.09	2.68	9.75	8.94	277	3.80	3.61	1.05	2.40	1.20	2.00	18	317.25±2.25
		8	16.58	2.68	9.75	9.26	300	3.80	3.67	1.04	2.52	1.24	2.03	19	327.0±4.5
		9	16.25	2.68	9.75	8.94	289	3.80	3.67	1.04	2.52	1.24	2.03	19	317.25±2.25
		10	16.09	2.52	9.26	8.78	253	3.67	3.54	1.04	2.36	1.16	2.03	20	314.0±1.0
		Mean	16.17	2.66	9.46	8.92	272.7	3.75	3.58		2.45	1.18		18.6	
		Standard Deviation	.26	.09	.23	.18	13.86	.05	.07		.02			1.20	

* Genetic line = A.

TABLE XI

T TEST PROBABILITY VALUES FOR COMPARISON OF STARVED AND NON-STARVED LARVAE USED FOR REARING QUEENS

Experiment	Treatment	Length of body	Length of tongue	Length of abdomen		Weight of pupa	Head		Basitarsus		Length of cell
				a[1]	b[2]		width	length	length	width	
4	Starvation vs Control	4.13 P<0.01 C+ Df=38	2.79 P<0.01 C+ Df=35	4.35 P<0.01 C+ Df=38	4.39 P<0.01 C+	2.91 P<0.01 C+	N.S. Df=39	N.S.	5.84 P<0.01 C+	N.S.	4.02 P<0.01 C+
6	Starvation vs Control	N.S. Df=14	N.S. Df=13	N.S. Df=14	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Legend: N.S. = Non Significant
Df = Degree of freedom
C = Control treatment
\+ = Treatment having the largest mean within an experiment.

CHAPTER VI

THE EFFECT ON ADULTS PRODUCED FROM YOUNG LARVAE REARED IN ONE, TWO, AND THREE DAY OLD QUEEN CELLS

In commercial queen rearing the removal of a worker larva from a one day old queen cell and its replacement with a younger larva, (i.e., "double grafting"), is often practised. However, no data about the merits of double grafting exist. Therefore a study was done to show the effect on the adult queens, of rearing them, from larvae placed in one, two, and three day old queen cells. The purpose of this study was to make recommendations (concerning correct queen rearing methods) available to the queen rearing industry and to obtain further information about the process of queen-worker differentiation if possible.

I. WEIGHT MEASUREMENTS OF ROYAL JELLY IN ONE, TWO, AND THREE DAY OLD QUEEN CELLS

INTRODUCTION

One of the differences observed between one, two, and three day old queen cells, containing larvae, was a progressive increase in the amount of royal jelly present in the cells, corresponding to an increase in the age of the cells. Therefore, two experiments were performed, to measure the weight of royal jelly in one, two, and three day old queen cells.

Methods and results

Two experiments were performed using the basic queen rearing methods reported in Chapter II, page 10. The same four hives and the progeny of the same breeder queen were used in both experiments. The larvae were grafted into plastic queen cell cups, each containing a small drop of royal jelly and distilled water (1:1). At the beginning of each experiment thirty six larvae under six hours of age (Chapter V, page 32), were grafted into each of the four hives.

At 24, 48 and 72 hours after grafting, about one-third of the number of cells originally accepted (containing living larvae) 24 hours after grafting, were removed each day from each of the four hives. The 24, 48 and 72 hour old larvae were removed from the cells and the combined weight of each cell and the royal jelly it contained, was recorded. The royal jelly was then removed from the cells with a small spatula; the small amount of royal jelly remaining was washed out with the aid of a gentle stream of water. The empty cells were allowed to dry by evaporation and then weighed. The weights of the royal jelly are shown in Table XII.

The data for both experiments 1 and 2 show that the weight of royal jelly present in queen cells increases significantly from 24 to 48 to 72 hours after grafting.

Discussion and conclusions

The weight of royal jelly present in one, two, and three day old queen cells increases with the age of the larva in the cell, up to a

period of 72 hours after grafting. This means that the amount of food initially available to young larvae grafted in one, two, or three day old queen cells (see Chapter VI, Section III), from which the original larvae have been removed, increases day by day up to 72 hours.

The possible effect of these different amounts of food being available to young larvae, as shown by the characteristics of the queens produced, is discussed more fully at the end of this chapter.

II. THE EFFECT OF REARING QUEENS FROM YOUNG LARVAE PLACED IN VARIOUS LOCATIONS IN QUEEN CELLS

INTRODUCTION

In preliminary experiments involved in placing young larvae in one, two and three day old queen cells, from which the original larvae had been removed, it was found that an indentation corresponding in size to the size of the larva removed, was left in the surface of the royal jelly in the cell. The jelly immediately beneath the indentation appeared to have a more watery consistency than the rest of the firmer, whiter, surrounding royal jelly in the cell, and may have been caused by the "puddling" effect of the larva in the cell. This difference was also noted earlier by von Rhein (1933). Therefore two tests were done to ascertain if the location in which a young larva is placed in the royal jelly in a cell during grafting (from which another larva had been removed) affects the external morphology of the pupae and adults produced from them.

Methods and results

The indentation left in the royal jelly when a one day old larva is removed from the cell is very small, while the indentation left when a three day old larva is removed from the royal jelly is correspondingly large, leaving little surrounding royal jelly. Therefore, cells from which two day old larvae had been removed from their royal jelly were used for the experiments, because a young larva could be placed accurately either in the indentation or the surrounding royal jelly.

Two experiments were done, each comparing the pupal and adult external morphology of bees produced from:

a) placing a young larva in the indentation ("Inside") left when a two day old larva was removed from its cell,

b) placing a young larva on the royal jelly surrounding ("Outside") the indentation,

c) placing a young larva on royal jelly, stirred ("Mix") after a two day old larva had been removed from its cell,

d) placing a young larva in a small drop of 1:1 royal jelly and distilled water mixture ("Control").

Experiments 1 and 2 were done consecutively in the same cell builder colony (a) using the basic queen rearing techniques (see Chapter II, page 10). Young larvae under six hours of age (see Chapter V, page 32), were used for all experiments. Larvae of genetic line A were used in experiment 1 and larvae of genetic line B were used in experiment 2.

There were four treatments in each experiment, namely, Inside, Outside, Mix, and Control (page 44), with the cells of each treatment

being distributed randomly on the bars in the supporting frames.

In both experiments 1 and 2, 72 larvae were grafted individually on a small drop of 1:1 royal jelly and water mixture in cell cups and then placed in the cell builder colony (a). In both experiments the cells were removed from the cell builder colony (a) 48 hours later; the two day old larvae were removed from the cells the bees had just fed ("accepted"). In experiment 1 (36 larvae, 9 per treatment) and experiment 2 (72 larvae, 18 per treatment), young larvae were then grafted to serve as controls or to replace the discarded two day old larvae. The young larvae (less than six hours old) then completed their development in cell builder colony (a) in both experiments.

Several days after capping the cells were removed from the hive to the incubator (see Chapter II, page 11), and measurements were done on the pupae and the adults produced from the larvae (see Chapter III).

Larval mortalities occurring on the different consistencies of royal jelly, i.e., Inside, Outside, Mix, and Control (Page 44) are shown in Table XIII. A morphological comparison of queens reared on royal jelly of different consistencies is given in Table XIV (experiment 1) and Table XV (experiment 2). The results in Table XVI show a significant difference ($P < 0.01$) between the length of cell in the control and each of the three treatments (Inside, Outside and Mix) in both experiments 1 and 2, with the exception that no significant difference was obtained between the control and mix treatment in experiment 1. A significant difference ($P < 0.05$) in the length of tongue between the Control and

and Inside treatment in experiment 2 was found. However none of the remaining characteristics measured, showed a significant difference between the control and each treatment in both experiments 1 and 2.

Discussion and conclusions

The non-significant T test probability values between controls and the other treatments (Inside, Outside and Mix) in Table XVI, for all characteristics (one exception) in both experiments 1 and 2, strongly suggests that during grafting it matters little where a larva is placed on the royal jelly in a two day old queen cell*. The one exception was a T test probability value ($P < 0.05$) in experiment 2, for the Inside - Control treatment.

The Inside, Outside and Mix treatments for the length of cell, gave significantly larger measurements than the control in both experiments (except for experiment 1, Mix - Control Treatment, which was non-significant and might be due to the low number of cells measured). The greater amount (depth) of royal jelly in the two day old queen cells as compared to the Control (a small drop of 1:1 royal jelly and distilled water), may cause the worker bees to build longer queen cells to accomodate the extra royal jelly in the Inside, Outside and Mix treatments.

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* See Discussions and conclusions, Chapter VI, Section III.

TABLE XII

WEIGHT OF ROYAL JELLY IN ONE, TWO, AND THREE DAY OLD QUEEN CELLS

Experiment	Hive No.	Mean weight of royal jelly (mgs) at:					
		24 hrs.	(No. of cells per treatment)	48 hrs.	(No. of cells per treatment)	72 hrs.	(No. of cells per treatment)
#1	1	71.41	(8)	210.23	(8)	475.21	(7)
	2	97.26	(5)	199.37	(6)	582.69	(7)
	3	73.22	(10)	200.14	(11)	534.59	(10)
	4	70.63	(6)	188.13	(6)	*	
#2	1	47.16	(8)	196.89	(8)	566.34	(9)
	2	53.11	(7)	189.36	(9)	532.89	(9)
	3	58.40	(3)	225.10	(4)	539.98	(5)
	4	*					

* Note: A mated queen escaped through the queen excluder in the cell finishing colony #4 resulting in non-acceptance of larvae in Experiment #1 at 72 hrs., and Experiment #2 at 24 hrs. after grafting.

TABLE XIII

A SUMMARY OF LARVAL MORTALITY OF QUEENS REARED ON DIFFERENT INITIAL CONSISTENCIES OF ROYAL JELLY

Experiment	Treatment	No. of larvae per treatment	Larval mortality at 1st. check (hrs)	Total larval mortality between grafting and capping	No. of cells capped at (hrs)	Total No. of adults produced
1	Inside a	9	2 (23)	3	6 (138.5)	6
	Outside b	9	0 (23)	1	8 (138.5)	8
	Mix c	9	3 (23)	4	5 (138.5)	5
	Control d	9	5 (23)	5	4 (138.5)	4
2	Inside a	18	2 (20.5)	7	11 (116.5)	11
	Outside b	18	0 (20.5)	4	14 (116.5)	13
	Mix c	18	4 (20.5)	10	8 (116.5)	8
	Control d	18	8 (20.5)	10	8 (116.5)	8

TABLE XIV

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM THE DIFFERENT PLACEMENTS OF YOUNG LARVAE IN TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm). a^1	b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
1.	(Inside)	1	16.41	2.44	9.59	9.10	287	3.80	3.54	1.07	2.56	1.20	2.13	20	300.5±4.5
		2	16.41	2.44	9.75	9.26	282	3.80	3.67	1.03	2.48	1.20	2.07	20	280.75±0.75
		3	16.90	2.52	10.24	9.91	276	3.73	3.61	1.03	2.48	1.20	2.07	18	275.5±4.5
		4	16.09	2.36	9.75	9.10	276	3.80	3.61	1.05	2.40	1.20	2.00	21	275.5±4.5
		5	16.25	2.44	9.43	9.10	280	3.80	3.54	1.07	2.40	1.20	2.00	20	300.5±4.5
		6	16.25	2.60	9.59	9.10	262	3.73	3.54	1.05	2.52	1.20	2.10	19	275.5±4.5
		Mean	16.39	2.47	9.73	9.26	277.2	3.78	3.59		2.48	1.20		19.7	

continued...

TABLE XIV (continued)

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
1.	(Outside)	1	16.25	2.68	9.75	8.94	298	3.86	3.73	1.03	2.60	1.24	2.10	19	300.5±4.5
		2	15.93	2.44	10.08	8.94	276	3.73	3.61	1.03	2.56	1.20	2.13	20	280.75±0.75
		3	16.09	2.44	9.43	8.94	277	3.73	3.54	1.05	2.52	1.20	2.10	19	300.5±4.5
		4	16.41	2.44	9.75	9.10	280	3.80	3.54	1.07	2.52	1.20	2.10	19	282.5±1.0
		5	16.41	2.44	9.75	9.10	251	3.67	3.48	1.05	2.40	1.12	2.14	20	275.5±4.5
		6	16.58	2.60	10.08	9.43	278	3.92	3.67	1.07	2.48	1.24	2.00	18	300.5±4.5
		7	16.09	2.44	9.75	8.94	271	3.80	3.54	1.07	2.44	1.20	2.03	19	280.75±0.75
		8	16.09	2.60	9.91	8.94	279	3.80	3.67	1.03	2.52	1.20	2.10	19	275.5±4.5
		Mean	16.23	2.51	9.81	9.04	276.3	3.79	3.60		2.51	1.20		19.1	

continued...

TABLE XIV (continued)

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
1.	(Mix)	1	16.58	2.44	9.91	9.43	284	3.73	3.61	1.03	2.56	1.20	2.13	22	300.5±4.5
		2	16.25	2.52	9.75	9.10	280	3.86	3.61	1.07	2.60	1.20	2.17	21	275.5±4.5
		3	16.25	2.44	9.59	9.10	275	3.80	3.54	1.07	2.44	1.20	2.03	19	300.5±4.5
		4	16.58	2.44	9.91	9.26	287	3.86	3.67	1.05	2.44	1.16	2.10	20	300.5±4.5
		5	17.06	2.44	10.08	9.75	304	3.86	3.67	1.05	2.64	1.24	2.13	18	300.5±4.5
		Mean	16.54	2.46	9.85	9.33	286.0	3.82	3.62		2.54	1.20		20.0	
1.	(Control)	1	16.09	2.44	9.91	9.10	273	3.80	3.61	1.05	2.48	1.20	2.07	16	275.5±4.5
		2	16.58	2.44	9.75	9.26	281	3.80	3.54	1.07	2.60	1.16	2.24	18	300.5±4.5
		3	16.09	2.44	9.75	8.94	262	3.73	3.54	1.05	2.48	1.16	2.14	15	280.75±0.75
		4	16.25	2.52	9.75	9.10	274	3.67	3.48	1.05	2.60	1.16	2.24	16	300.5±4.5
		Mean	16.25	2.46	9.79	9.10	272.5	3.75	3.54		2.54	1.17		16.3	

TABLE XV

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM THE DIFFERENT PLACEMENTS OF YOUNG LARVAE IN TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
2.	(Inside)	1	15.76	2.68	8.78	8.61	273	4.05	3.80	1.07	2.56	1.24	2.06	18	305.75±0.25
		2	15.76	2.68	9.26	8.61	280	3.92	3.73	1.05	2.64	1.24	2.13	17	309.75±0.75
		3	15.93	2.68	9.10	8.61	272	3.92	3.80	1.03	2.52	1.24	2.03	18	305.75±0.25
		4	15.93	2.76	8.78	8.45	278	3.92	3.80	1.03	2.52	1.20	2.10	18	306.5±0.5
		5	15.76	2.68	9.26	8.61	271	3.92	3.67	1.07	2.52	1.24	2.03	18	301.0±4.5
		6	16.41	2.68	9.75	9.10	282	3.99	3.80	1.05	2.44	1.20	2.03	16	305.75±0.25
		7	15.93	2.76	8.61	8.61	250	3.92	3.67	1.07	2.48	1.24	2.00	18	305.75±0.25
		8	16.58	2.60	9.43	9.26	289	3.99	3.80	1.05	2.60	1.20	2.17	17	325.5±4.5
		9	16.58	2.68	9.43	9.10	284	3.92	3.67	1.07	2.56	1.24	2.06	17	301.0±4.5
		10	16.41	2.60	9.59	9.10	300	3.99	3.73	1.07	2.52	1.20	2.10	19	325.5±4.5
		11	16.41	2.68	9.43	9.10	293	3.99	3.73	1.07	2.68	1.28	2.09	17	308.0±1.0
		Mean	16.13	2.68	9.22	8.83	279.3	3.96	3.75		2.55	1.23		17.6	

continued...

TABLE XV (continued)

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
2.	(Outside)	1	16.41	2.60	9.43	9.10	281	3.99	3.73	1.07	2.52	1.24	2.03	18	305.75±0.25
		2	15.76	2.60	9.10	8.45	271	3.92	3.73	1.05	2.56	1.24	2.06	18	325.5±4.5
		3	15.93	2.68	9.10	8.61	284	3.99	3.80	1.05	2.52	1.24	2.03	19	311.25±0.75
		4	16.41	2.60	9.75	9.26	291	3.92	3.73	1.05	2.60	1.20	2.17	18	301.0±4.5
		5	16.09	2.52	9.26	8.78	272	3.92	3.67	1.07	2.56	1.20	2.13	19	305.75±0.25
		6	15.93	2.68	8.78	8.61	263	3.86	3.67	1.05	2.52	1.24	2.06	19	310.75±1.25
		7	16.58	2.76	9.59	9.26	285	3.92	3.80	1.03	2.64	1.24	2.13	19	325.5±4.5
		8	15.76	2.60	8.94	8.45	273	3.92	3.73	1.05	2.52	1.20	2.10	19	301.0±4.5
		9	16.25	2.60	9.43	8.94	291	3.92	3.80	1.03	2.60	1.24	2.10	18	301.0±4.5
		10	16.09	2.76	9.10	8.61	288	3.92	3.80	1.03	2.48	1.24	2.00	17	325.5±4.5
		11	16.09	2.68	9.26	8.78	278	3.86	3.73	1.03	2.48	1.20	2.07	18	309.75±0.75
		12	15.76	2.68	8.94	8.45	264	3.80	3.67	1.03	2.48	1.16	2.14	18	325.5±4.5
		Mean	16.09	2.65	9.22	8.78	278.4	3.91	3.74		2.54	1.22		18.3	

continued...

TABLE XV (continued)

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
2.	(M 1 x)	1	16.41	2.76	9.75	9.26	301	3.92	3.73	1.05	2.52	1.20	2.10	20	325.5±4.5
		2	16.25	2.68	9.43	8.94	278	3.99	3.80	1.05	2.56	1.24	2.06	20	325.5±4.5
		3	16.41	2.60	9.59	9.10	286	3.99	3.80	1.05	2.52	1.20	2.10	19	325.5±4.5
		4	16.09	2.68	9.10	8.78	267	3.86	3.67	1.05	2.60	1.24	2.10	18	301.0±4.5
		5	15.76	2.52	8.94	8.45	279	3.86	3.61	1.07	2.52	1.24	2.03	18	325.5±4.5
		6	16.58	2.68	9.59	9.26	290	3.86	3.67	1.05	2.60	1.20	2.17	20	331.5±1.5
		7	16.58	2.60	9.59	9.26	275	3.99	3.73	1.07	2.56	1.24	2.06	18	337.0±1.0
		8	16.58	2.68	9.59	9.26	289	3.92	3.73	1.05	2.52	1.20	2.10	18	325.5±4.5
		Mean	16.33	2.65	9.45	9.04	283.1	3.92	3.72		2.55	1.22		18.9	

continued...

TABLE XV (continued)

Experiment	Treatment	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
2.	(Control)	1	16.25	2.68	9.26	8.94	278	3.92	3.73	1.05	2.64	1.24	2.14	17	331.5±1.5
		2	16.41	2.60	9.10	8.94	277	3.86	3.67	1.05	2.56	1.20	2.13	17	325.5±4.5
		3	16.09	2.52	9.10	8.78	275	3.92	3.73	1.05	2.52	1.24	2.03	16	325.5±4.5
		4	15.93	2.68	8.94	8.45	280	3.99	3.80	1.05	2.52	1.24	2.03	16	325.5±4.5
		5	16.41	2.52	9.26	9.10	272	3.92	3.61	1.09	2.52	1.20	2.10	15	*
		6	16.25	2.68	9.26	8.94	287	3.99	3.80	1.05	2.56	1.24	2.07	16	301.0±4.5
		7	16.09	2.68	8.94	8.61	272	3.99	3.80	1.05	2.48	1.24	2.00	17	311.25±0.75
		8	16.41	2.60	9.10	8.78	288	3.99	3.73	1.07	2.60	1.24	2.10	17	325.5±4.5
	Mean		16.23	2.62	9.12	8.82	278.6	3.95	3.73		2.55	1.23		16.4	

*Failed to emerge completely.

TABLE XVI

A SUMMARY OF T TEST PROBABILITY VALUES FOR QUEENS REARED ON DIFFERENT INITIAL CONSISTENCIES OF ROYAL JELLY

Experiment	Treatment	Pupal Measurements					Adult Measurements				Length of capped cell (mm)
		Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Width of head (mm)	Length of head (mm)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	
#1 (Line A)	Inside control	N.S. Df=8	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	4.72 P<0.01
	Outside control	N.S. Df=10	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	5.39 P<0.01
	Mix control	N.S. Df=7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
#2 (Line B)	Inside control	N.S. Df=17	2.11 P<0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	3.19 P<0.01
	Outside control	N.S. Df=18	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	6.23 P<0.01
	Mix control	N.S. Df=14	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	5.70 P<0.01

Legend: N.S. = Non significant
Df. = Degrees of freedom

III. THE EFFECT ON QUEENS OF REARING THEM FROM YOUNG LARVAE PLACED IN ONE, TWO, AND THREE DAY OLD QUEEN CELLS

INTRODUCTION

The conclusions that: (1) the weight of royal jelly present in one, two, and three day old queen cells increases with the age of the larvae in the cell up to a period of 72 hours after grafting, (Chapter VI, Section I.) and that (2) there is no significant difference between Control, Inside, Outside, and Mix treatments (Chapter VI, Section II), for almost all of the pupal and adult characteristics measured laid the foundation for this study. In the following investigation the grafting of young larvae into one, two, and three day old queen cells and the measurements of the resulting pupae and adults was done:

1) to obtain data about the practise of "double grafting" (the placing of young larvae under six hours old into one day old queen cells).

2) to obtain data about the effect of grafting young larvae into two and three day old queen cells in an effort to gain further information about the process of queen - worker differentiation.

Methods and results

The basic queen rearing techniques described in Chapter II, (page 10), were used in all experiments in this section. Larvae from the

same breeder queen[1] were placed in the same cell builder colonies[2] in all treatments within each individual experiment. In the control treatment for all experiments (1-11 inclusive), young larvae were placed on a small drop of 1:1 royal jelly and distilled water mixture. In experiments 1-9 inclusive, coloured tacks and/or coloured pencils were used to randomly mark, on the bars opposite the cells, the designation of the treatment each cell was to receive.

PART I

A preliminary set of experiments (1-5 inclusive) were done to compare control treatments with young larvae grafted into one, two and three day old queen cells. In each experiment, an excess number of young larvae were grafted on day 1. On day 2[3], those cells accepted by the bees from the graft on day 1, were consolidated (placed in rows on the bars of the frames and the unaccepted cells removed). The control larvae remained untouched after the first large graft on day 1. On each subsequent day (days 2, 3, and 4), the one, two, or three day old larvae in the cells, corresponding to the designated treatment, were removed and replaced with young larvae. The number of larvae per treatment, number of larvae capped, and number of adults that emerged were recorded for

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[1]Exception:
Experiment 1, the grafting of young larvae into a three day old queen cell. (See Table XVII).

[2]Exception:
Experiments 10 and 11. (See page 62).

[3]"Days" were considered 24 hours apart.

each of experiments 1-5 inclusive, and these data are shown in Table XVII. A summary of the combined data of experiments 1 to 5 is given in Table XVIII.

The results show that very few of the larvae placed in three day old queen cells became adult queens and emerged from their cells (Tables XVII and XVIII). The results (Table XVIII) also show that more bees died between the time of capping and the time of emergence (mortality in the post-capping stages) in the control treatment[4] than in treatments on which young larvae were placed in one and two day old queen cells. Because of considerable rainy and overcast weather which results in unsatisfactory rearing conditions (i.e., the bees cannot forage for food and the colony is more disturbed than normally, by the opening of the hive) during the early summer (July 5--July 23), when the experiments were done, it was possible that the control larvae in particular, received insufficient quantities of food for a period of time after grafting. This may have resulted in the larval and post-capping mortality in the control treatments being greater than normal. The removal of the frames from the cell builder colonies for grafting on days 2, 3, and 4, also exposed the control larvae (on three successive days) to conditions outside the hive more often than the other three treatments. This may have increased the larval mortality and the mortality in the post-capping stages in the control treatments more than it affected mortality in treatments in which larvae were grafted into one, two, and three day old queen cells.

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[4]For all experiments in this section, a larvae of the control treatment = a young larvae placed on a small drop of 1:1 royal jelly and distilled water mixture in a cell.

PART II

A series of four experiments (6-9 inclusive) were done next, to further test the effect of grafting young larvae into one and two day old queen cells. The grafting of young larvae into three day old queen cells was omitted from experiments 6-9 inclusive, because of the low numbers of adults produced in experiments 1 to 5 (Part I, page 58), and because of the practical limitations involved, (i.e., obtaining adequate numbers of larvae under six hours of age, and of grafting the young larvae into cells before the larvae and/or the royal jelly in the cells dries to some degree).

Instead of grafting in sequence and having one, two, three, and four day old larvae present in a cell building colony at one time, a different method was used: on day 1, young larvae were grafted and placed into Colony (A), and on day 2, young larvae were grafted and placed into Colony (B). On day 3 the frames, containing the cells, were removed from Colonies A and B and the one and two day old larvae were removed from the cells. The larvae were replaced with young larvae and the one and two day old cells were arranged randomly on the bars with the cells of the control treatment, and then placed in Colony (C).

Three different genetic lines, A, B, and C, (see Chapter IV, page 23) were used throughout the series of experiments 6-9 inclusive, with two of the three genetic lines being used in each experiment. In each treatment within an experiment, one half of the larvae used were of

one genetic line and one-half of the larvae use of another genetic line[5].

A total of 36 larvae, 12 larvae per treatment, were grafted on day 3 in each experiment. Extra larvae were also grafted and placed in Colony D, to be used as replacements, in the event that early larval mortality (non-acceptance) might occur in Colony C. A summary of the larval acceptance, time of capping, and post-capping mortality is given for experiments 6 to 9 in Table XIX.

After the cells were capped in each experiment they were removed to the incubator (see Chapter II, page 11) and later the pupal and adult measurements were done and recorded in Tables XX, XXI, XXII and XXIII. The data in Tables XX, XXI, XXII and XXIII were statistically analyzed using the "unpaired T test", and the significant T test probability values appear in Table XXIV. The results in Table XXIV show that there is no significant difference (one exception) in the characteristics of pupae and adults measured, between the control, one day, and two day old queen cell treatments. The one exception was a significant difference ($P < 0.01$) between pupal weight of the control treatment and the two day old queen cell treatment in experiment 6.

A significant difference (see Table XXIV) between the control and the other two treatments was found between the lengths of cells, in experiments 6-9 inclusive[6]. When a significant difference was found in the

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[5]The control treatments in experiments 6-9 inclusive were statistically analyzed in the Chapter IV Genetic Studies.

[6]Exception:
Non significance between the control and one day old queen cell treatment (double grafting) in experiment 7 and 8.

length of cells between treatments, the value of the mean was larger in the two day old cell treatment than in the one day old cell treatment. Similarly, the value of the mean in the one day old queen cell treatment was larger than the control treatment. (See Tables XX, XXI, XXII, XXIII, and XXIV.).

PART III

It was found (Tables XVII and XVIII) that very few young larvae, which were placed in three day old queen cells, developed into adult bees and emerged from their cells. Therefore a preliminary study was done, to produce sufficient numbers (for statistical analysis of pupal and adult measurements) of queens reared from young larvae placed in three day old queen cells, in order to compare them to control treatments. Several attempts to rear young larvae in three day old queen cells were made early in the queen rearing season (July 11-15) but without success.

Later, in the queen rearing season (July 26 -- August 5), when the cell building colonies were very populous with bees (a prerequisite for queen rearing) experiments 10 and 11 were done consecutively to compare queens produced from control treatments to queens produced from larvae grafted into three day old queen cells.

Cell builder colonies A and B were used in each experiment. In experiment 10 the control larvae were placed in Colony A; the larvae grafted into the three day old queen cells were placed in Colony B. In experiment 11 the treatments were reversed, so that the larvae for the control treatment were placed in Colony B and the larvae grafted

in three day old queen cells were placed in Colony A. In both experiments 10 and 11, 72 larvae were grafted on day 1 into Colonies A (experiment 10) and B (experiment 11). On day 4, 72 hours later, the three day old queen cells were taken from the cell builder colonies A (experiment 10) and B (experiment 11). The three day old larvae were replaced with larvae less than 6 hours old and the bars and frames supporting the cell cups were returned to Colonies A (experiment 10) and B (experiment 11). At the same time control larvae were grafted and placed in the other colony: Colony B (experiment 10) and Colony A (experiment 11). Each treatment was placed in a separate cell builder colony in order to eliminate any preference the bees might have for feeding or caring for one treatment in a different way than the other, should both treatments be placed in the same colony.

The number of larvae per treatment, larval mortality, and number of adults produced appear in Table XXV, for each treatment in both experiments 10 and 11. The results indicate that mortality of larvae, placed in three day old queen cells, does not become serious until about 24 hours after grafting.

Discussion and conclusions

The study of the effect on adults produced from young larvae reared in one, two, and three day old queen cells was divided into three related sections.

First (Section I), the royal jelly present in one, two, and three day old queen cells was weighed and was found to increase with the age of the larvae in the cells up to a period of 72 hours after grafting. Therefore, there is a larger quantity of food initially available to a young larva with each 24 hour increase in the age of the cell (up to 72 hours) in which it is placed. (Table XII page 47).

Next (Section 2), the effects on queens produced from larvae reared in various locations (Inside, Outside and Mix treatments) within two day old queen cells, were tested, and there were no significant differences between the control and the other treatments for all pupal and adult characteristics measured. (Table XVI page 56). However, the length of the cells in the Inside, Outside, and Mix treatments were found to be significantly larger ($P < 0.01$) than the length of cells in the control treatments.

Possible explanations for the results in Section 2, were reenforced by the observations made, and the results recorded in Section 3, (see page 66).

In Section 3, Part I, a series of preliminary experiments (1 to 5), showed that very few of the young larvae used in grafts in three day old queen cells reached the adult stage and emerged from their cells (Tables XVII and XVIII). The high larval mortality in some of the control

treatments (example Table XVII, experiment 3) might have been caused by the unsatisfactory queen rearing conditions caused by rainy and overcast weather, and by the fact that the controls were exposed to conditions outside the hive more often than the other treatments (experiments 1 to 5).

Therefore the experiments were modified (Section 3, Part II) to eliminate these problems and then no significant difference (one exception), was found between the various pupal and adult characteristics measured for bees produced from larvae of the control treatment and from larvae placed in one, and two day old queen cells. The lengths of capped cells were also found to be significantly longer in two day old queen cell treatments than the cells of the control treatment (see page 66 for explanation).

In Section 3, Part III, experiments 10 and 11 were done to compare control treatments to that of queens reared from larvae placed in three day old queen cells. Of special interest was the observation that the young larvae placed in three day old queen cells did not suffer high larval mortality until about 24 hours after grafting.

Several interesting explanations can be made for the results obtained from the entire study of the effect on adult bees of rearing them from young larvae placed in one, two, and three day old queen cells.

The data (Tables XVI and XXIV show that capped queen cells from treatments in which young larvae are placed in one and two day old queen cells, are significantly longer* ($P < 0.01$) than control treatments. This

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*Two exceptions -- see Table XXIV.

was thought to be due to the progressively greater amounts of food found in the one, two, and three day old queen cells (Chapter VI, Section I).

From observation it appeared as if the young larvae placed in two or three day old queen cells, received little or no additional royal jelly for a period of time up to about 24 hours. Sometimes the young larvae placed in three day old queen cells appeared to remain several days before being fed fresh food, during which time the royal jelly in the cells tended to dry out and become hardened. However, when the bees started feeding the larvae in the two or three day old cells, the total depth and amount of royal jelly (fresh on top of old, drier royal jelly) increased in direct proportion to the age of the cell used in the treatment (up to 72 hours of age). Thus, the bees built longer cells to contain the greater depths of royal jelly found with each successive increase in the age of the cell used for a treatment.

It was also observed that in some experiments (1-5, and 10 and 11), in which young larvae were placed in three day old queen cells and poorly fed for a period of time after grafting, that after several days in the cell the larvae sometimes move to the side of the queen cell and became positioned partially out of the royal jelly. This caused the bees to extend the queen cells, to contain the larvae. Mortality resulted if the larva fell out of the royal jelly or stuck to the sides of the queen cell cup. There are several reasons why a young larva might tend to move downwards and partially or wholly out of the three day old royal jelly in its cell. Qualitative factors and/or the consistency of three day old royal jelly might make it unpalatable to a larva or impossible for a larva to

feed, or perhaps the consistency of the three day old royal jelly might be too solid for the young larva to remain (physically) in the royal jelly.

At least one possible explanation exists for the non significant differences in pupal and adult characteristics measured for control larvae and young larvae grafted into one and two day old queen cells. The control larvae have small amounts of diluted royal jelly surrounding them immediately after grafting, while the young larvae grafted into one and two day old queen cells have an abundance of food surrounding them. However, as time progresses, the control larvae receive an abundance of food while the larvae placed in two day old cells (and possibly one day old cells), must feed for a period of time before they receive fresh food from the nurse bees, on top of the royal jelly that is beginning to dry out. Thus the larvae in all treatments may have a period of development when they receive insufficient nutrition for proper growth, resulting in non significant differences between the pupal and adult characteristics measured.

The results in Section II (Tables XX - XXIV inclusive), strongly suggest that there is no significant difference in the pupal and adult characteristics measured for young larvae placed in one day old queen cells ("double grafting") and young larvae reared in control cells.

TABLE XVII

THE NUMBERS OF LARVAE GRAFTED, CELLS CAPPED AND ADULTS PRODUCED FROM CONTROL TREATMENTS AND YOUNG LARVAE PLACED IN ONE, TWO AND THREE DAY OLD QUEENS

Experiment	Treatment	Total No. of larvae grafted	Total No. of cells capped	Total No. of bees produced
#1	Control	27	26	22
	One day cell	27	25	15
	Two day cell	27	20	9
	Three day cell	27	5	3
#2	Control	18	18	6
	One day cell	18	13	10
	Two day cell	18	18	14
	Three day cell	18	6	4
#3	Control	9	6	1
	One day cell	9	9	9
	Two day cell	9	4	3
	Three day cell	9	2	1
#4	Control	9	9	6
	One day cell	9	8	4
	Two day cell	9	3	3
	Three day cell	9	0	0
#5	Control	9	9	2
	One day cell	9	5	2
	Two day cell	9	9	4
	Three day cell	9	5	2

TABLE XVIII

A SUMMARY OF THE NUMBER OF LARVAE GRAFTED, CELLS CAPPED AND ADULTS PRODUCED FROM CONTROL TREATMENTS AND YOUNG LARVAE PLACED IN ONE, TWO AND THREE DAY OLD QUEEN CELLS

Experiments	Treatment	Total No. of larvae grafted	Total No. of cells capped	Total No. of bees produced
1-5	Control	72	68	37
(combined)	One day cells	72	60	40
	Two day cells	72	54	33
	Three day cells	72	18	10

TABLE XIX

A SUMMARY OF LARVAL MORTALITY AND TIME OF CAPPING OF CELLS OF QUEENS REARED FROM LARVAE PLACED IN ONE, TWO AND THREE DAY OLD QUEEN CELLS

Experiment	Treatment	No. of larvae per treatment	Larval mortality at the times indicated (hrs)				Total No. of larvae replaced	No. of cells capped at times indicated (hrs)			Total No. of adults produced
#6	Control	12	0(21)	0(45)	1(68.5)	4(93.0)	4	12(103.5)			12
	One day cell	12	0(21)	0(45)	0(68.5)	3(93.0)	2	11(103.5)			11
	Two day cell	12	3(21)	3(45)	4(68.5)	3(93.0)	0	7(103.5)	8(117)		8
#7	Control	12	4(21.5)	4(45)			4	1(93.5)	9(98)	12(117)	12
	One day cell	12	0(21.5)	0(45)			0	7(93.5)	8(98)	12(117)	11
	Two day cell	12	0(21.5)	1(45)			1	5(93.5)	9(98)	12(117)	11
#8	Control	12	0(21.5)	0(50.5)	0(69.5)		0	2(93.5)	6(98.5)	12(117.5)	12
	One day cell	12	0(21.5)	0(50.5)	0(69.5)		0	0(93.5)	12(98.5)	12(117.5)	11
	Two day cell	12	0(21.5)	0(50.5)	1(69.5)	2(93.5)	2	4(93.5)	7(98.5)	12(117.5)	12
#9	Control	12	0(21.5)	0(50.5)	0(69.5)		0	5(95.5)	9(98.5)	12(119.5)	12
	One day cell	12	1(21.5)	1(50.5)	1(69.5)		1	7(95.5)	8(98.5)	12(119.5)	12
	Two day cell	12	0(21.5)	0(50.5)	2(69.5)		2	6(95.5)	10(98.5)	12(119.5)	10

TABLE XX

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM YOUNG LARVAE PLACED IN ONE AND TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
6	Control	A	1	15.60	2.44	9.26	8.78	256	3.67	3.61	1.02	2.52	1.20	2.10	13	307.5±4.5
		A	2	15.60	2.60	9.43	8.61	268	3.80	3.67	1.04	2.52	1.20	2.10	16	287.0±0.5
		A	3	15.93	2.76	9.10	8.61	254	3.86	3.67	1.05	2.56	1.24	2.06	14	279.0±4.5
		A	4	15.60	2.68	9.43	8.61	269	3.80	3.67	1.04	2.52	1.24	2.03	14	279.0±4.5
		A	5	15.76	2.44	8.61	8.45	269	3.86	3.67	1.05	2.52	1.24	2.03	14	307.5±4.5
		A	6	15.93	2.60	9.26	8.94	245	3.80	3.61	1.05	2.56	1.24	2.06	13	285.0±1.5
		B	7	16.09	2.68	9.10	8.78	281	3.92	3.80	1.03	2.64	1.24	2.13	14	307.5±4.5
		B	8	16.41	2.60	9.43	8.94	281	3.99	3.80	1.05	2.68	1.28	2.09	15	307.5±4.5
		B	9	16.09	2.60	9.10	8.78	275	3.86	3.80	1.02	2.64	1.24	2.13	14	307.5±4.5
		B	10	16.41	2.68	9.43	9.10	284	3.99	3.80	1.05	2.64	1.24	2.13	14	307.5±4.5
		B	11	16.09	2.76	8.94	8.78	275	3.92	3.80	1.03	2.72	1.28	2.13	15	307.5±4.5
		B	12	16.25	2.68	9.10	8.94	280	3.92	3.80	1.03	2.60	1.24	2.10	14	307.5±4.5
		Mean		15.98	2.63	9.18	8.78	269.8	3.87	3.73		2.59	1.24		14.2	

continued...

TABLE XX (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
6	One day old queen cells	A	1	16.09	2.60	9.10	8.94	268	3.86	3.73	1.03	2.64	1.28	2.06	17	287.0±0.5
		A	2	16.09	2.60	9.26	8.78	265	3.80	3.67	1.04	2.48	1.16	2.14	17	279.0±4.5
		A	3	16.09	2.76	9.43	8.78	279	3.86	3.67	1.05	2.60	1.24	2.10	15	279.0±4.5
		A	4	16.41	2.44	9.75	9.26	277	3.73	3.67	1.02	2.56	1.20	2.13	17	288.5±1.0
		A	5	16.41	2.76	9.43	9.10	283	3.92	3.73	1.05	2.60	1.20	2.17	15	307.5±4.5
		A	6	16.25	2.76	9.26	8.94	295	3.99	3.86	1.03	2.56	1.28	2.00	17	307.5±4.5
		B	7	16.09	2.76	9.26	8.78	271	3.86	3.67	1.05	2.64	1.28	2.06	15	307.5±4.5
		B	8	15.93	2.76	8.94	8.78	280	3.99	3.80	1.05	2.56	1.28	2.00	16	307.5±4.5
		B	9	16.09	2.76	9.26	8.78	289	3.92	3.73	1.05	2.64	1.28	2.08	15	307.5±4.5
		B	10	15.60	2.84	8.94	8.61	259	3.86	3.67	1.05	2.52	1.28	1.97	15	279.0±4.5
		B	11	16.09	2.60	9.26	8.78	272	3.92	3.73	1.05	2.60	1.28	2.03	17	307.5±4.5
		Mean		16.10	2.69	9.26	8.87	276.2	3.88	3.72		2.58	1.25		16.0	

continued...

TABLE XX (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
6	Two day old queen cells	A	1	16.25	2.60	9.43	9.26	285	3.86	3.67	1.05	2.64	1.24	2.13	17	279.0±4.5
		A	2	16.25	2.60	9.43	8.94	262	3.73	3.61	1.03	2.60	1.20	2.17	19	287.0±0.5
		A	3	15.76	2.44	8.78	8.78	265	3.86	3.54	1.09	2.56	1.24	2.06	17	288.5±1.0
		A	4	16.25	2.76	9.59	9.26	279	3.80	3.73	1.02	2.48	1.20	2.07	17	288.5±1.0
		B	5	16.25	2.76	9.43	8.78	285	3.92	3.80	1.03	2.56	1.32	1.94	18	307.5±4.5
		B	6	16.41	2.76	9.43	8.94	282	3.92	3.80	1.03	2.60	1.28	2.03	17	307.5±4.5
		B	7	16.25	2.84	9.43	9.10	279	3.92	3.73	1.05	2.64	1.24	2.13	16	279.0±4.5
		B	8	16.09	2.76	9.43	9.10	286	3.99	3.80	1.03	2.48	1.24	2.00	17	307.5±4.5
		Mean		16.19	2.69	9.37	9.02	277.9	3.88	3.71		2.57	1.25		17.3	

TABLE XXI

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM YOUNG LARVAE PLACED IN ONE AND TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
7	Control	B	1	15.76	2.76	8.78	8.61	277	3.92	3.80	1.03	2.56	1.20	2.13	14	386.25±1.25
		B	2	15.93	2.60	9.10	8.78	273	4.05	3.80	1.07	2.64	1.20	2.20	14	278.5±4.5
		B	3	15.93	2.68	9.26	8.61	274	3.92	3.80	1.03	2.68	1.28	2.09	15	289.75±1.25
		B	4	16.09	2.76	9.10	8.94	266	3.99	3.73	1.07	2.52	1.24	2.03	13	278.5±4.5
		B	5	16.09	2.60	9.26	8.61	275	3.99	3.73	1.07	2.64	1.24	2.13	14	284.0±1.0
		C	6	15.76	2.68	9.10	8.60	276	3.86	3.61	1.07	2.64	1.20	2.20	14	289.75±1.25
		C	7	16.41	2.76	9.43	9.26	290	3.92	3.73	1.05	2.72	1.20	2.27	14	284.0±1.0
		C	8	16.25	2.76	9.10	8.94	278	3.86	3.73	1.03	2.60	1.20	2.17	14	278.5±4.5
		C	9	16.25	2.68	9.75	8.94	291	3.99	3.73	1.07	2.64	1.20	2.20	15	302.0±4.5
		C	10	16.41	2.84	9.26	9.10	294	3.92	3.67	1.07	2.64	1.24	2.13	13	302.0±4.5
		C	11	16.41	2.68	9.26	9.10	308	3.99	3.73	1.07	2.56	1.20	2.13	14	289.75±1.25
		Mean		16.12	2.71	9.22	8.86	282.0	3.95	3.73		2.62	1.22		14.0	

Continued...

TABLE XXI (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
7	One day old queen cells	B	1	15.44	2.76	9.10	9.59	264	3.92	3.67	1.07	2.56	1.24	2.06	16	289.75±1.25
		B	2	16.25	2.84	9.26	8.94	281	4.05	3.80	1.07	2.60	1.24	2.10	16	278.5±4.5
		B	3	16.25	2.68	9.26	8.78	280	3.99	3.80	1.05	2.72	1.28	2.13	18	278.5±4.5
		B	4	15.93	2.84	9.26	8.61	265	3.99	3.73	1.07	2.68	1.28	2.09	16	278.5±4.5
		B	5	15.76	2.60	9.10	8.61	254	3.92	3.67	1.07	2.48	1.20	2.07	16	278.5±4.5
		C	6	16.58	2.76	9.59	9.43	298	3.99	3.73	1.07	2.52	1.28	1.97	18	292.0±0.5
		C	7	16.58	2.92	9.91	9.43	312	3.92	3.67	1.07	2.64	1.08	2.44	16	292.0±0.5
		C	8	16.25	2.76	9.43	8.94	312	3.99	3.80	1.05	2.76	1.24	2.23	15	302.5±4.5
		C	9	16.90	2.60	9.59	9.43	285	3.92	3.61	1.09	2.60	1.16	2.24	17	386.25±1.25
		C	10	16.25	2.76	9.75	8.78	300	4.05	3.73	1.09	2.52	1.20	2.10	16	289.75±1.25
		C	11	15.76	2.68	9.10	8.45	284	3.99	3.73	1.07	2.56	1.20	2.13	15	386.25±1.25
		Mean		16.18	2.75	9.40	9.00	285.0	3.98	3.72		2.60	1.22		16.3	

continued...

TABLE XXI (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a^1	Length of abdomen (mm) b^2	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
7	Two day old queen cells	B	1	16.58	2.84	9.59	9.10	294	3.99	3.86	1.03	2.64	1.24	2.13	18	302.5±4.5
		B	2	15.93	2.68	9.26	8.78	278	3.99	3.67	1.09	2.60	1.20	2.17	17	284.0±4.5
		B	3	16.58	2.60	9.59	9.26	279	3.99	3.80	1.05	2.64	1.24	2.13	17	278.5±4.5
		B	4	16.41	2.60	9.26	9.10	274	3.99	3.80	1.05	2.56	1.24	2.06	15	278.5±4.5
		B	5	15.76	2.60	9.26	8.78	266	3.92	3.73	1.07	2.60	1.20	2.17	17	278.5±4.5
		B	6	16.09	2.68	9.10	8.94	264	3.99	3.73	1.07	2.56	1.20	2.13	18	278.5±4.5
		C	7	16.09	2.68	9.26	8.78	284	3.99	3.80	1.05	2.52	1.24	2.03	17	278.5±4.5
		C	8	16.41	2.92	9.43	9.10	291	3.92	3.73	1.07	2.64	1.24	2.13	17	291.25±0.25
		C	9	16.41	2.76	9.75	9.10	307	3.99	3.73	1.07	2.56	1.24	2.06	17	289.75±1.25
		C	10	16.25	2.68	9.59	8.94	296	3.92	3.67	1.07	2.68	1.24	2.16	17	284.0±4.5
		C	11	16.09	2.76	9.10	8.61	286	3.92	3.73	1.05	2.56	1.24	2.06	18	289.75±1.25
		C	12	16.90	2.76	9.91	9.59	289	3.92	3.67	1.07	2.72	1.16	2.34	16	278.5±4.5
		Mean		16.29	2.71	9.43	9.01	284.0	3.96	3.74		2.61	1.22		17.0	

TABLE XXII

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM YOUNG LARVAE PLACED IN ONE AND TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Genetic line	Bee No	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development. (hrs)
8	CONTROL	A	1	16.41	2.60	9.28	9.10	267	3.86	3.67	1.05	2.56	1.16	2.21	14	304.0±4.5
		A	2	16.58	2.44	9.75	9.43	278	3.86	3.67	1.05	2.56	1.20	2.13	16	304.0±4.5
		A	3	16.58	2.60	9.59	9.26	283	3.80	3.67	1.04	2.68	1.16	2.31	14	304.0±4.5
		A	4*						3.73	3.61	1.03	2.56	1.20	2.13	15	278.5±4.5
		A	5	16.41	2.76	9.43	9.10	270	3.86	3.67	1.05	2.52	1.24	2.03	15	304.0±4.5
		A	6	16.25	2.52	9.59	9.10	265	3.73	3.67	1.02	2.56	1.20	2.13	15	304.0±4.5
		C	7	16.25	2.44	9.59	9.10	285	3.80	3.67	1.04	2.60	1.16	2.24	15	304.0±4.5
		C	8	16.90	2.44	10.24	9.59	305	3.92	3.80	1.03	2.76	1.20	2.30	15	328.0±4.5
		C	9	16.90	2.60	10.08	9.75	292	3.92	3.80	1.03	2.60	1.20	2.17	15	304.0±4.5
		C	10	16.58	2.44	9.91	9.26	281	3.92	3.73	1.05	2.56	1.20	2.13	15	304.0±4.5
		C	11	16.90	2.44	10.08	9.59	286	3.86	3.73	1.04	2.64	1.16	2.28	14	304.0±4.5
		C	12	16.58	2.44	9.59	9.26	290	3.92	3.67	1.07	2.60	1.16	2.24	14	310.0±1.5
	Mean			16.58	2.52	9.74	9.32	282.0	3.85	3.70		2.60	1.19		14.8	

*Ready to emerge before pupa could be measured.

Continued...

TABLE XXII (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head Index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
8	One day old queen cells	A	1	16.25	2.68	9.26	8.94	272	3.86	3.67	1.05	2.52	1.20	2.10	16	304.0±4.5
		A	2	16.41	2.44	9.75	9.26	274	3.86	3.67	1.05	2.56	1.16	2.21	15	304.0±4.5
		A	3	16.41	2.44	9.91	9.26	277	3.80	3.67	1.04	2.64	1.20	2.20	15	304.0±4.5
		A	4	16.25	2.44	9.59	9.10	274	3.86	3.67	1.05	2.48	1.16	2.14	14	304.0±4.5
		A	5	16.58	2.44	9.75	9.26	271	3.73	3.54	1.05	2.52	1.16	2.17	16	304.0±4.5
		A	6	16.41	2.60	9.75	9.26	266	3.73	3.61	1.03	2.52	1.20	2.10	15	213.75±1.75
		C	7	17.06	2.44	10.08	9.75	303	3.92	3.80	1.03	2.56	1.20	2.13	16	213.75±1.75
		C	8	17.06	2.44	10.24	9.75	287	3.99	3.67	1.09	2.60	1.20	2.17	16	304.0±4.5
		C	9	16.41	2.60	9.59	9.10	314	4.05	3.86	1.05	2.68	1.20	2.23	16	328.0±4.5
		C	10	17.23	2.44	10.40	9.91	310	3.86	3.73	1.04	2.64	1.20	2.20	16	304.0±4.5
		C	11	17.06	2.60	10.24	9.75	292	3.80	3.67	1.04	2.48	1.16	2.14	14	304.0±4.5
	Mean			16.65	2.51	9.87	9.39	285.5	3.86	3.69		2.56	1.19		15.4	

Continued...

TABLE XXII (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
8	Two day old queen cells	A	1	15.76	2.68	9.10	8.45	264	3.86	3.67	1.05	2.48	1.20	2.07	18	304.0±4.5
		A	2	16.58	2.68	9.91	9.26	300	3.80	3.73	1.02	2.64	1.24	2.13	18	313.75±1.75
		A	3	16.58	2.52	11.05	9.43	274	3.67	3.61	1.02	2.56	1.20	2.13	19	304.0±4.5
		A	4	16.41	2.52	9.75	9.10	275	3.80	3.73	1.02	2.60	1.16	2.24	19	304.0±4.5
		A	5	16.09	2.52	9.26	8.94	260	3.86	3.67	1.05	2.64	1.16	2.28	19	304.0±4.5
		A	6	16.58	2.60	9.91	9.43	272	3.80	3.67	1.04	2.64	1.20	2.20	17	304.0±4.5
		C	7	16.58	2.60	9.75	9.26	296	3.99	3.80	1.05	2.60	1.24	2.10	19	313.75±1.75
		C	8	15.93	2.44	9.10	8.94	257	3.73	3.61	1.03	2.60	1.20	2.17	19	304.0±4.5
		C	9	17.06	2.44	10.40	9.75	305	4.11	3.86	1.07	2.72	1.24	2.19	17	304.0±4.5
		C	10	16.58	2.60	9.75	9.26	299	3.99	3.80	1.04	2.68	1.16	2.31	18	310.5±1.5
		C	11	16.41	2.60	9.75	9.10	280	3.80	3.61	1.06	2.52	1.20	2.10	18	304.0±4.5
		C	12	16.90	2.44	9.75	9.59	301	3.92	3.73	1.05	2.64	1.20	2.20	17	328.0±4.5
	Mean			16.46	2.55	9.79	9.21	281.9	3.86	3.71		2.61	1.20		18.2	

TABLE XXIII

A MORPHOLOGICAL COMPARISON OF QUEENS REARED FROM YOUNG LARVAE PLACED IN ONE AND TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
9	Control	A	1	16.90	2.68	10.08	9.75	272	3.80	3.67	1.03	2.64	1.20	2.37	16	305.5±4.5
		A	2*						3.92	3.80	1.03	2.60	1.20	2.17	14	284.5±4.5
		A	3*						3.86	3.67	1.05	2.52	1.28	1.97	15	284.5±4.5
		A	4	16.25	2.44	9.91	9.26	270	3.73	3.54	1.05	2.60	1.24	2.07	15	284.5±4.5
		A	5	16.74	2.44	10.40	9.75	251	3.80	3.61	1.05	2.56	1.16	2.21	14	284.5±4.5
		A	6	16.25	2.44	9.59	8.94	273	3.73	3.67	1.02	2.68	1.24	2.16	15	305.5±4.5
		B	7	16.90	2.44	10.56	10.08	265	3.86	3.73	1.03	2.56	1.28	2.00	16	300.5±6.0
		B	8	16.58	2.60	9.91	9.43	270	3.92	3.67	1.07	2.56	1.16	2.21	17	292.5±0.5
		B	9	16.58	2.84	9.75	9.10	282	4.05	3.86	1.05	2.60	1.24	2.07	17	305.5±4.5
		B	10	16.25	2.68	9.59	8.94	280	3.99	3.80	1.05	2.68	1.24	2.16	15	305.5±4.5
		B	11	16.41	2.68	10.08	9.26	276	3.86	3.73	1.03	2.52	1.20	2.10	15	305.5±4.5
		B	12	16.41	2.76	9.75	8.94	300	4.05	3.92	1.03	2.72	1.28	2.13	14	305.5±4.5
	Mean			16.53	2.60	9.96	9.35	273.9	3.88	3.72		2.60	1.23		15.3	

*Ready to emerge before pupa could be measured.

continued...

TABLE XXIII (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
9	One day old queen cells	A	1	16.09	2.52	10.40	9.10	266	3.73	3.61	1.03	2.56	1.20	2.13	17	284.5±4.5
		A	2	16.74	2.60	10.56	9.91	268	3.80	3.67	1.03	2.56	1.24	2.06	17	284.5±4.5
		A	3	16.74	2.60	10.56	9.91	254	3.67	3.54	1.04	2.56	1.12	2.29	18	289.25±1.25
		A	4	16.25	2.60	9.75	8.94	270	3.92	3.73	1.05	2.56	1.20	2.13	16	302.0±4.5
		A	5	16.90	2.52	10.24	9.59	288	3.92	3.73	1.05	2.64	1.24	2.13	17	289.25±1.25
		A	6	16.25	2.52	10.24	9.10	272	3.80	3.61	1.05	2.68	1.24	2.16	18	292.5±0.5
		B	7	16.58	2.76	10.08	9.10	286	3.99	3.86	1.03	2.60	1.20	2.17	17	302.0±4.5
		B	8	16.58	2.44	9.75	9.59	256	3.86	3.67	1.05	2.48	1.24	2.00	17	284.5±4.5
		B	9	16.74	2.76	10.24	9.59	284	3.99	3.73	1.07	2.64	1.12	2.36	17	305.5±4.5
		B	10	16.41	2.76	9.43	9.10	277	3.92	3.73	1.05	2.52	1.20	2.10	17	305.5±4.5
		B	11	16.25	2.68	9.43	8.94	263	3.86	3.67	1.05	2.52	1.24	2.03	17	305.5±4.5
		B	12	16.41	2.44	10.24	9.59	267	3.86	3.73	1.03	2.52	1.24	2.03	17	284.5±4.5
		Mean		16.50	2.60	10.08	9.37	270.9	3.86	3.70		2.57	1.21		17.1	

continued...

TABLE XXIII (continued)

Experiment	Treatment	Genetic line	Bee No.	Pupal Measurements: Length of body (mm)	Length of tongue (mm)	Length of abdomen (mm) a[1]	Length of abdomen (mm) b[2]	Weight of pupa (mgs)	Adult Measurements: Width of head (mm)	Length of head (mm)	Head index (W/L)	Length of basi-tarsus (mm)	Width of basi-tarsus (mm)	Basi-tarsal index (L/W)	Length of capped cell (mm)	Length of development (hrs)
9	Two day old queen cells	A	1	16.58	2.44	11.21	9.43	279	3.73	3.54	1.05	2.64	1.24	2.13	18	284.5±4.5
		A	2	15.93	2.60	10.08	8.78	267	3.80	3.67	1.03	2.52	1.20	2.10	19	289.25±1.25
		A	3	16.90	2.44	10.24	9.59	281	3.86	3.67	1.05	2.52	1.24	2.06	18	392.25±0.25
		A	4	16.90	2.60	10.73	9.75	281	3.86	3.61	1.07	2.64	1.20	2.20	18	290.75±0.25
		B	5	16.09	2.68	9.75	9.10	281	3.92	3.80	1.03	2.64	1.24	2.13	19	292.5±0.5
		B	6	16.09	2.60	9.59	8.94	268	3.86	3.73	1.03	2.60	1.28	2.03	19	302.0±4.5
		B	7	16.09	2.60	9.10	8.78	276	3.92	3.73	1.05	2.56	1.24	2.06	20	302.0±4.5
		B	8	16.25	2.68	10.08	8.94	273	3.92	3.73	1.05	2.64	1.20	2.20	18	291.5±0.5
		B	9	16.25	2.68	9.91	9.10	267	3.86	3.73	1.03	2.52	1.20	2.10	19	289.25±1.25
		B	10	16.09	2.36	10.08	9.10	265	3.92	3.73	1.05	2.52	1.20	2.10	20	284.5±4.5
	Mean			16.32	2.57	10.08	9.15	273.8	3.87	3.69		2.58	1.22		18.8	

TABLE XXIV

T TEST PROBABILITY VALUES FOR COMPARISON OF BEES PRODUCED FROM CONTROL TREATMENTS AND FROM LARVAE PLACED IN ONE OR TWO DAY OLD QUEEN CELLS

Experiment	Treatment	Length of body	Length of tongue	Length of abdomen a[1]	Length of abdomen b[2]	Weight of pupa	Head Width	Head Length	Basitarsus Length	Basitarsus Width	Length of cell
#6	Control/One day cell	N.S. Df=20	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	4.79 ($P<0.01$) C-
	Control/Two day cell	N.S. Df=19	N.S.	N.S.	N.S.	6.88 C- ($P<0.01$)	N.S.	N.S.	N.S.	N.S.	7.80 ($P<0.01$) C-
#7	Control/One day cell	N.S. Df=21	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Control/Two day cell	N.S. Df=22	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	9.51 ($P<0.01$) C-
#8	Control/One day cell	N.S. Df=21	N.S.	N.S.	N.S.	N.S.	N.S. Df=22	N.S.	N.S.	N.S.	N.S.
	Control/Two day cell	N.S. Df=22	N.S.	N.S.	N.S.	N.S.	N.S. Df=23	N.S.	N.S.	N.S.	3.42 ($P<0.01$) C-
#9	Control/One day cell	N.S. Df=20	N.S.	N.S.	N.S.	N.S.	N.S. Df=23	N.S.	N.S.	N.S.	5.08 ($P<0.01$) C-
	Control/Two day cell	N.S. Df=19	N.S.	N.S.	N.S.	N.S.	N.S. Df=21	N.S.	N.S.	N.S.	4.02 ($P<0.01$) C-

Legend: N.S.= non significant.
Df = degrees of freedom.
C- = control has the smallest mean within an experiment.

TABLE XXV

MORTALITY OF LARVAE PLACED IN THREE DAY OLD QUEEN CELLS

Experiment	Treatment	No. of larvae per treatment	Larval mortality at (hrs) after grafting					Total No. of adults produced
#10	Control	48	25(23.5)	27(48.5)	27(68.5)			20
	Three day cell	60	1(23.5)	41(48.5)	50(68.5)	53(92.5)	54(116.5)	6
#11	Control	36	11(19.5)	14(93.5)	20(67.5)	25(91.5)		11
	Three day cell	36	1(19.5)	16(93.5)	19(67.5)	22(91.5)		12

CHAPTER VII

GENERAL DISCUSSION AND CONCLUSIONS

To better understand the factors affecting queen rearing, experiments were done in this study in such a way as to eliminate as many variables as possible. However in commercial queen rearing techniques, a combination of factors may be interacting at any given time. Therefore when making recommendations to the queen producer, about the best queen rearing procedures to follow, one must take into consideration the particular set of conditions with which he is working. In commercial queen rearing, the effects on the adult bees produced:

1) from larvae of different genetic lines;

2) from larvae starved prior to grafting; and

3) from larvae placed in one day old queen cells ("double grafting"), may be such that an interaction occurs between one or more of these factors.

The pupal and adult characteristics of bees produced from larvae of different genetic lines, are probably affected to a large degree by the various conditions present, i.e., the starvation of young larvae prior to grafting, or the placing of young larvae into one, two, and three day old queen cells. In Chapter V, experiments 4 and 6, the significant difference found between most characteristics measured for the starvation and control treatments in experiment 6, and the non significant difference between the same treatments in experiment 4, could be largely due to the ability of larvae to starve for short periods prior to grafting, a

characteristic inherent in the different genetic lines (A or B) used in the experiment.

One of the secondary objectives of this thesis was to elucidate, where possible, the mechanism of queen - worker differentiation. Although little data is available concerning queen - worker differentiation, most authors agree that queen - worker differentiation is influenced either by a qualitative or quantitative difference (or both) in the larval food. The grafting of young larvae into one, two, and three day old queen cells (Chapter VI, Section 3), presented the young larvae with different quantities and possibly different qualities of food.

Rhein (1933) found that the extent of ovary development had no correlation with body size, and that the royal jelly used in his incubator tests did not seem to have some factor necessary for queen determination. He concluded that the amount of food ingested by a larva could not be the decisive factor in queen - worker differentiation. Haydak (1943) postulated that only the richly nourished queen larvae acquire fully developed ovaries, and that these secrete enough hormones to produce secondary sex characteristics. Rhein (1951) stated that the formation of a queen necessitated two phases of feeding, a) with young queen larval brood food so that the larva was "predetermined", and b) with old queen larval brood food when the larva weighed about 20 mg; so that the larva was finally "determined".

Johansson (1955), in reviewing the works of other authors, listed the following criticisms of the qualitative differential mechanism.

i) glandular bee food is variable in composition,

ii) the composition of glandular secretions cannot be changed at will by bees,

iii) it is not possible to assume, as does Gontarski (1949), that the different age classes of nurse bees provide qualitatively different foods to larvae of various ages and sexes, since Lindauer (1952), has shown that each nurse bee seems to feed larvae of all ages.

Shuel and Dixon (1960), reviewed most of the previous literature on queen - worker differentiation and ~~they~~ their work can be summarized briefly as follows:

1. Development in the worker larva occurs in two distinct phases, delimited by the addition of honey to the diet by the nurse bees around the third or fourth day of the larval stadium.

2. The dichotomy between castes appears to be initiated during the first phase and consummated in the second. There is little doubt that nutrition was the major extrinsic factor in caste establishment. The identity of the dietary factor initiating the series of events culminating in female dimorphism has not yet been established, and results of many rearing experiments strongly suggest the involvement of a substance which is either volatile or unstable:

Shuel and Dixon (1960), also stated that the mode of action of the dietary factor likewise was unknown and suggested that a difference in hormonal balance between castes is established in early larval life and is the intermediary factor linking nutrition to dimorphism.

Habowsky and Shuel (1959), found that there was no qualitative difference between the protein fractions of royal jelly and the worker diet which could account for female dimorphism.

Smith (1959), concluded that food quality as well as quantity should be considered in respect to differentiation, because a worker larva normally is never without an excess of food. He also stated that pollen is not a prerequisite to queen - worker differentiation and said that moisture, protein and fat contents increase in the food of older queen larvae but decrease in the food of older worker larvae.

Simpson (1961), thought that the differentiating factor must be sought in something that operated throughout the feeding life of the larva rather than in a change of diet at a particular age. He said that although underfeeding in queen cells could produce workers, over-feeding in worker cells would not produce queens. Abundance of food was necessary for development of the queen but it would not of itself cause queen development. He concluded that if differentiation is brought about by a difference in the feeding of the two castes, it must be a qualitative difference.

To date no real evidence has been presented to demonstrate the exact nature of the mechanism of queen - worker differentiation. This indeed provides a tantalizing area of research.

It is possible that with further research, the grafting of young larvae into one, two, and three day old queen cells could be used as a tool for investigating queen - worker differentiation. If the royal jelly present at the time of grafting and the royal jelly fed to young larvae after grafting were chemically analyzed, as well as weights of royal jelly

taken before grafting and at intervals after grafting young larvae into one, two, and three day old queen cells and these data correlated with measurements of pupae and adults produced, further information about the process of queen - worker differentiation may be found.

On the basis that there was no significant difference between most pupal and adult morphological characteristics of queens reared from control larvae, and larvae grafted into one day old queen cells, (i.e., "double grafting", see page 60, experiments 6-9 inclusive), it is suggested that under normal conditions "double grafting" serves little purpose. "Double grafting" requires more work, as the queen producer must perform one extra graft and about an extra day is required to produce an adult queen. Time factors are critical in the spring for the queen rearing industry. However if starvation of young larvae prior to grafting or starvation of the larvae after grafting is a possibility, then the extra food present in a one day old cell might give better acceptance and produce pupae and adults with larger external measurements. (See Chapter VI, Section 3, Part I).

The study of the external morphological measurements of pupae and adult bees, reared from larvae subjected to different treatments is only the first in what should eventually become a several stage study. It now remains for someone to measure the internal morphological characteristics (example: diameter of the spermatheca and the number of ovarioles per ovary) of pupae and/or adult bees produced from the different treatments*.

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*All adult bees measured in this thesis were preserve in F.A.A. solution, in the event that internal morphological measurements wish to be taken.

The final step involves the comparison of the external and internal morphological characteristics of pupae and/or adults produced with their performance, (example: egg laying ability, rate of colony build up, size of workers produced, honey production, etc.) as queens in the hive. Only then can it be conclusively stated that any one treatment produces higher quality queens.

CHAPTER VIII

SUMMARY

Rearing studies of queen honey bees were done using a basic queen rearing system. One box breeder colonies were used to provide young larvae for grafting; cell builder colonies, with the queen confined to the bottom box, were used to rear the larvae and to cap the cells.

Measurements were taken of the external morphology of pupae and adults produced. The characteristics measured were chosen on the basis of simplicity of measurement, degree of accuracy, and value as indicators of dimorphic differentiation.

Experiments were done in which three genetic lines were compared. The results showed that a significant difference occurs between genetic lines for most of the external morphological characteristics measured. The weight of the pupa was considered to be one of the best measurements for differentiating between different genetic lines.

The effect of starvation, as shown by the external characteristics of pupae and adults reared from young larvae starved six hours prior to grafting, was tested. One experiment showed no significant difference between the control (larva grafted on to 1:1 royal jelly and distilled water mixture) and the starvation treatment, while a second experiment showed a significant difference between the control and the starvation treatment for most of the characteristics measured.

A series of experiments were also done to study the effect of placing young larvae in one, two, and three day old queen cells.

First, the weight of royal jelly exclusive of the larvae, was measured in one, two, and three day old queen cells. A significant increase in the weight of royal jelly in one, two, and three day old queen cells was found.

Next, the effect of placing young larvae in various locations on royal jelly in queen cells, as shown by the external characteristics of the pupae and adults produced, was tested. There was no significant difference in most of the external characteristics of the pupae and adults measured for the control treatment and those of the larvae placed on the "Inside", "Outside", or "Mixed" royal jellies in the queen cells.

A series of experiments were then done to compare the pupal and adult characteristics of bees produced from control treatments to those of queens produced from larvae placed in one, two, and three day old queen cells. The number of adults produced per number of young larvae grafted into three day old queen cells was very small. Further experiments were done to compare both pupae and adults reared from control treatments and from larvae placed in one and two day old queen cells. In most cases there was no significant difference in the pupal and adult characteristics measured between the three treatments. Two later experiments were done to compare the effect on larvae that were placed in three day old queen cells to the effect on larvae in control treatments. It was observed that when young larvae were placed in three day old queen cells, the royal jelly in the cells became partially dried, with little or no

food being fed to the larvae by the worker bees for at least 24 hours after grafting. Many of the young larvae placed in three day old queen cells tended to move out of the royal jelly in the cell and this phenomenon was thought to be at least partly responsible for the high mortality. The unpalatability and/or the dryness of the royal jelly may have prevented the larvae from feeding and therefore they moved out of the royal jelly in search of food. The consistency of the royal jelly may also have been such that it was physically impossible in some cases for the larvae to remain suspended at the top of their cells in the dry royal jelly.

There was no significant difference in most cases, in the pupal and adult characteristics measured for bees produced from control treatments and bees produced from placing young larvae in one day old queen cells ("double grafting"). It is therefore suggested that under normal rearing conditions, "double grafting", which requires extra work for the queen producer, does not produce significantly higher quality queens than those reared from control treatments.

It is also suggested that the internal morphological measurements and studies of the queens' performance in the hive, should be compared with the external morphological measurements of bees reared under the same conditions. Only then can the factors affecting queen rearing be fully understood.

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